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CLINICAL AND EXPERIMENTAL

DECREASED ABSORPTION FROM THE ALIMENTARY TRACT FOLLOWING INJECTION OF POSTERIOR PITUITARY EXTRACT*

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IN THE treatment of a case of poisoning, one of the aims is to decrease or delay absorption of the poison from the alimentary tract. To this end the physician washes the stomach, and gives purgatives and precipitating antidotes. Occasionally one may give an antidote which destroys unabsorbed poison in the stomach and intestine, such as permanganate for alkaloidal poisons. None of these procedures is entirely satisfactory. Therefore, an additional means of delaying and decreasing absorption should be welcome. Such a means is the subcutaneous injection of the extract of the posterior lobe of the hypophysis (Liquor Pituitarii, U.S.P.). The experiments of Rees[1] and Douglas,[2] conclusively proved that pituitary extract decreases absorption from isolated intestinal loops in anesthetized animals. In view of the uncertainty of transfer of these results to unanesthetized, intact animals, the following experiments were carried out.

INDIRECT EXPERIMENTS

Strychnine.—The first experiments, begun eight years ago, were concerned with indirect evidence of delayed and decreased absorption. A series of four rabbits were given 0.00315 gm. per kg. of strychnine sulphate by stomach tube, and the time of onset and intensity of symptoms noted. A few days later, the dose was repeated, but with a subcutaneous injection of 0.25 c.c. of obstetric pituitary extract, twenty minutes and five minutes before the ad-

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ministration of the strychnine. In the case of three of these rabbits, the interval between the administration of strychnine and the onset of hyperexcitability was two to three times as long when preceded by pituitary extract subcutaneously as in the control experiments. A fourth animal failed to show any delay in onset of hyperexcitability with pituitary extract. The experiments were repeated on the first three rabbits, but in reverse order, the pituitary experiment being first tried, and the control experiment two or three days later. Again the symptoms were delayed when the oral administration of strychnine was preceded a few minutes by the injection of liquor pituitarii.

Finally, two rabbits were injected with liquor pituitarii as before, and were given a larger dose of strychnine (0.00325 gm. per kg.). Hyperexcitability occurred in both rabbits. A few days later, the same rabbits were given strychnine alone. Both had convulsions and died. Table I summarizes two typical experiments.

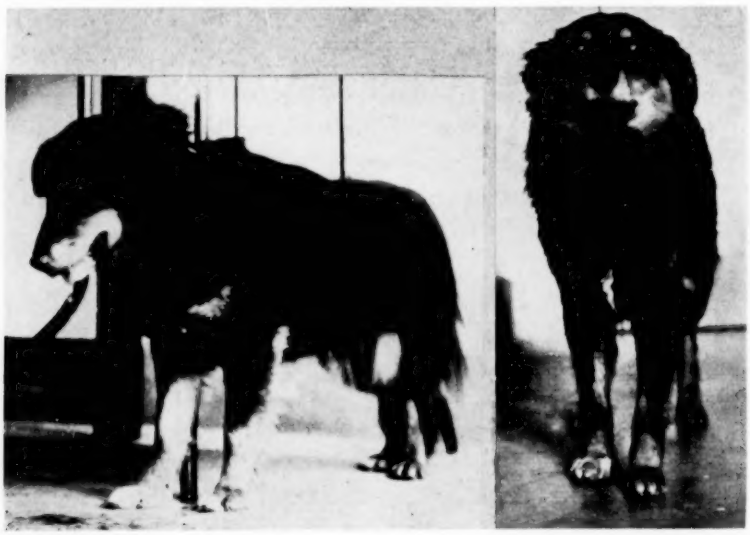

Fig. 1.—Effect of posterior pituitary extract upon response of dog to orally administered morphine. Left: control fifty minutes after morphine. Note salivation and swaying of hind quarters. Right: inject with pituitary extract. Note alert appearance and absence of drooling.

From these experiments it was evident that the injection of extract of the posterior lobe of the hypophysis afforded a definite protection against orally administered strychnine.

Morphine.—A similar delay and decrease in symptoms from the oral administration of morphine occurred when the animals were injected with liquor pituitarii. Because of the resistance of rabbits to oral doses of morphine (200 mg. per kg. failed to depress), dogs were used. In the control experiments, two dogs received 4 mg. per kg. of morphine sulphate by stomach tube while two others received injections of 0.5 c.c. acidulated NaCl solution fifteen minutes and five minutes before the administration of the morphine. The NaCl solution was acidulated to the P_H of the ampoules of pituitary solution.* A

*In control experiments with all other drugs, similar injections of acidulated salt solution were employed.

week later the administration of morphine was preceded fifteen minutes and five minutes by injections of 0.5 c.c. pituitary solution. A few days later, the pituitary-morphine experiments were repeated, and still later the control experiments with morphine.

In the control experiments symptoms of medullary stimulation were evident within four to ten minutes (nausea, salivation, retching, vomiting, panting), and symptoms of cerebral depression (drowsiness, unsteadiness, and swaying of hind quarters) in from fifteen to thirty-five minutes. In the pituitary experiments, there was little or no evidence of medullary stimulation; a slight unsteadiness occurred at the end of about thirty-five minutes, and swaying only at the end of an hour. Fig. 1 contrasts the symptoms in dog II, fifty minutes after morphine administration, in the control and pituitary periods. Note in the control, the blurring of the hind quarters, due to swaying, and the relaxation of muscles allowing exaggerated angulation of the joints of the forepaws. That the delay and decrease in symptoms of morphine action was probably due to delayed absorption is supported by the fact that pituitary extract did not influence the action of *subcutaneously* injected morphine in tests on 3 dogs.

TABLE I

EFFECT OF POSTERIOR PITUITARY EXTRACT UPON THE RESPONSE OF RABBITS TO ORALLY ADMINISTERED STRYCHNINE

DATE	DOSE PER KG.	TIME OF ONSET	SYMPTOMS
	Rabbit VI		
Nov. 21, 1922	0.00315 with pituitrin	33 min.	Hyperexcitability
Nov. 23, 1922	0.00315 control	10 min.	Hyperexcitability
Nov. 27, 1922	0.00315 with pituitrin	40 min.	Hyperexcitability
Nov. 30, 1922	0.00315 control	18 min.	Hyperexcitability
	Rabbit VII		
Nov. 21, 1922	0.00325 with pituitrin	60 min.	Hyperexcitability
Nov. 23, 1922	0.00325 control	22 min.	Hyperexcitability
		40 min.	Convulsions
		52 min.	Death

Cannabis.—The dose of fluid extract of cannabis, found to cause uniform effects in dogs when given in soft capsule, was 0.05 c.c. per kg. The symptoms used in judging the degree of depression, and the average time of appearance in control tests were (a) excitement, barking, twenty to thirty minutes; (b) "far-away look," thirty to thirty-five minutes; (c) drooping of head, fifty to sixty minutes; (d) swaying, fifty to seventy-five minutes. Pituitary solution, injected before cannabis was given, resulted in a decrease in the severity of the symptoms and a delay of from twenty to sixty-five minutes in their appearance.

Iodide.—Five-tenths gram of potassium iodide in 25 c.c. water was given to each of eight rabbits by stomach tube. Two human subjects each took 0.3 gram KI, the one in 250 c.c. water, the other in capsule without water. Urine was collected from rabbits by catheter, from the human subjects by voluntary voiding. Salivary excretion of iodide also was studied in man. The total excretion of the iodide by the rabbits over a two-hour period was 17.5 per cent less in the pituitary experiments than in the controls.

In the experiments with human subjects, the differences between control and pituitary periods were more striking than in the rabbit experiments. During the control the average time of appearance of iodide in the urine and saliva were eighteen minutes and ten minutes respectively, as compared with seventy-five minutes and fifty minutes in the pituitary periods. The average two-hour urinary excretion of iodide (calculated as KI) in the control periods was 24.9 mg. as compared with 10.6 mg. in the pituitary periods; the average salivary excretions were 9.9 mg. and 4.9 mg. respectively. Fig. 2 illustrates a typical experiment.

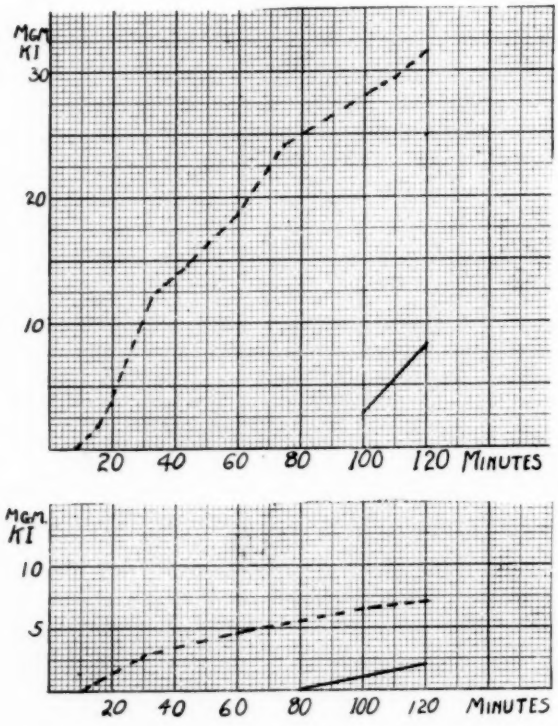

Fig. 2.—Urinary and salivary excretion of iodide in human subject. Upper chart, urinary excretion: lower chart, salivary excretion. Broken line, control period: unbroken line, pituitary period.

Further support of delayed iodide absorption resulting from injections of pituitary extract is given by the direct experiments on rats, described later in this paper.

Glucose.—A striking effect of pituitary solution in retarding absorption of glucose was shown in the following experiments. Glucose was given by stomach tube to each of five rabbits, and blood-sugar concentrations determined fifteen minutes after the glucose administration and at thirty-minute intervals for three to four hours thereafter. The dose of glucose was 1.75 gm. in 2.5 c.c. water per kg. The Shaffer-Hartmann method was used in the determination of blood sugar. In the control trials there was a marked rise in the blood-sugar curve, the apex usually being reached in one and a half hours. In the

pituitary experiments, the blood-sugar rise was much less than in the controls, except in one instance. In this instance, however, the peak came an hour later than in the control. The curves in the pituitary trials were closely paralleled by curves after the administration of one-half the usual dose (0.875 gm. per kg.) but without pituitary solution, indicating that the rate of absorption

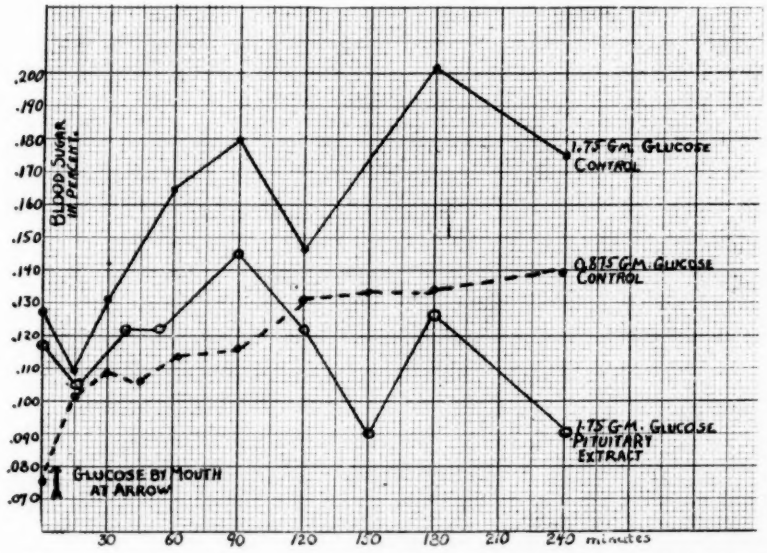

Fig. 3.—Blood-sugar curves following administration of glucose by stomach tube in rabbit.

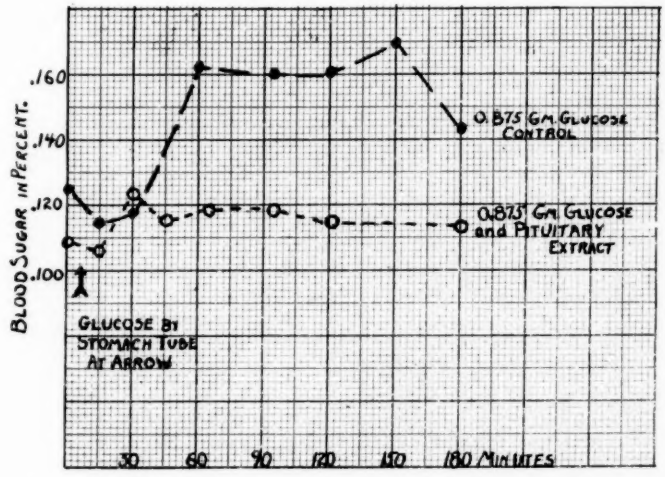

Fig. 4.—Blood-sugar curves following administration of glucose to rabbit by stomach tube.

following pituitary injections was about one-half that of the control period. This is confirmed by the direct experiments on rats, reported below. Fig. 3 shows the blood-sugar curves of a typical animal.

Blood-sugar curves, following 0.875 gm. glucose per kg., with and without pituitary injections, were determined on one rabbit, with the result shown in

Fig. 4. It is evident that there was a marked decrease in the rate of glucose absorption when the rabbit received pituitary solution subcutaneously. That the lower blood-sugar curves in the pituitary injected animals were probably not due to an effect of the gland extract upon absorbed glucose is indicated by the experiments of Hines, Leese and Boyd.[3] These investigators observed that during slow intravenous infusion of glucose the blood-sugar curves were higher when pituitary extract was injected, than in the control. The experiments of several other investigators give further support to this view.[4, 5, 6]

DIRECT EXPERIMENTS

In view of the fact that the above data gave only indirect evidence of decreased absorption, following injections of postpituitary extract, the direct method on the rat, devised by Cori and Cori,[7] was employed. This was considered necessary, especially with iodide and glucose because of the well-known effects of the pituitary hormones upon kidney function, and because of some uncertainty as to their effect on glucose metabolism. Koref and Mautner,[8] using this method, showed definite delay in absorption of water.

Briefly, the method consisted in the administration to rats, by stomach tube, of 2 c.c. of 25 per cent glucose or iodide per 100 gm. body weight, preceded twenty minutes and five minutes respectively by subcutaneous injections of 0.125 c.c. pituitary extract, or acidulated salt solution (controls). At the

TABLE II

GLUCOSE ABSORPTION. HOURLY ABSORPTION OF GLUCOSE FROM THE ALIMENTARY TRACT OF RATS. NOTE DIFFERENCE BETWEEN CONTROL RATS AND RATS RECEIVING INJECTIONS OF PITUITARY EXTRACT. MILLIGRAMS GLUCOSE ABSORBED HOURLY PER 100 GRAMS BODY WEIGHT

	CONTROLS	TEST RATS INJECTED WITH PITUITARY
	190	140
	222	169
	200	94
	262	143
	192	135
	195	91
	184	96
Average	206	125

TABLE III

IODIDE ABSORPTION. HOURLY ABSORPTION OF IODIDE FROM THE ALIMENTARY TRACT OF RATS. NOTE THE DIFFERENCE BETWEEN THE CONTROL RATS AND RATS RECEIVING INJECTIONS OF PITUITARY EXTRACT. MILLIGRAMS POTASSIUM IODIDE ABSORBED HOURLY PER 100 GRAMS BODY WEIGHT

	CONTROLS	TEST RATS INJECTED WITH PITUITARY
	86	60
	102	30
	110	43
	127	37
	98	80
	96	76
Average	103	54

end of two hours, the animals were killed by a blow on the occiput, the esophagus and the rectum tied off and the entire gastrointestinal tract removed, opened in distilled water, repeatedly rinsed, and the glucose or iodide content of the filtrate determined. The Schaffer-Hartmann method for glucose, and the dichromate-H_2SO_4-carbontetrachloride-thiosulphate method for iodide were employed. The rats weighed from 125 gm. to 215 gm., with the majority between 180 gm. and 200 gm. Six rats were used in each series. The sexes were equally distributed between the control and test groups. Tables II and III clearly show that the rate of absorption in the rats receiving pituitary solution was about one-half that of the controls.

SUMMARY

Subcutaneous injection of reliable commercial extracts of the posterior lobe of the hypophysis resulted in a decrease in the rate of absorption of five chemically dissimilar drugs from the gastrointestinal tract, as evidenced by

1. A delay of onset and decrease in symptoms produced by strychnine sulphate administered by stomach tube to rabbits, and by morphine sulphate (stomach tube) and fluid extract of cannabis (soft capsule) in dogs.

2. Failure of pituitary solution to alter the response of dogs to morphine sulphate administered subcutaneously.

3. Decreased urinary and salivary excretion of orally administered iodide in man and rabbits.

4. Decrease in rise of blood sugar from orally administered glucose in rabbits.

5. An increase in the amount of glucose and of iodide remaining in the gastrointestinal tract of rats two hours after administration of the respective substances by stomach tube.

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ASSAY OF TINCTURES OF DIGITALIS*

A Cross-section Survey of Metropolitan Boston

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THE object of this survey is to determine to what extent tincture of digitalis, as dispensed in representative drug stores of Metropolitan Boston, conforms in strength to the standard laid down as official in the United States Pharmacopoeia.

Although this is, strictly speaking, a local survey the results are none the less of general interest, owing to the fact that more than 75 per cent of the tinctures assayed are the products of commercial pharmaceutical houses, whose tinctures of digitalis are depended upon by practicing physicians throughout the country.

Data are also presented pertaining to the potency of the former U. S. P. tincture, the present U. S. P. tincture, chain drug store tinctures, and locality of purchase.

All the samples investigated were purchased over the counter and note was made, when information was available, regarding the manufacturer, the age of the tincture, and whether or not it was fat-free.

That the strength of digitalis varies within a wide range is common knowledge. Hence a complete review of the literature is unnecessary. A brief summary of the literature of results of tincture assay follows:

Bennefeld[1] in 1881 was the first to appreciate the importance of tincture standardization and to assay digitalis physiologically, determining the minimal lethal dose in terms of body weight. His research was carried out upon six tinctures secured from different parts of Germany, and his results show a wide variation range in tincture strength, i.e., 100 per cent to 400 per cent. Realizing the importance of the points made by Bennefeld, considerable research followed in perfecting satisfactory methods of standardization.

Frankel,[2] in 1902, using the one-hour frog method, assayed 6 tinctures obtained in and around Heidelberg. His results also show a variation of from 100 per cent to 400 per cent. Two years later Barger and Shaw,[3] working with a set of nine weak samples obtained from commercial sources, found that the potency of the strongest tincture was one and one-half times that of the weakest. Tinctures of low potency might be expected to yield lower variation changes. Dixon and Haynes,[4] in 1906, analyzed mixed commercial samples not all of recent origin, and described variations up to 180 per cent. Edmunds,[5] in 1907, standardized 17 samples, obtained on the open market

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from different parts of this country, by the one-hour frog method, and found great variations. Even among samples from the same source his variations ran as high as 400 per cent. Edmunds and Hale,[6] in 1908, studied the variability of four tinctures using three methods (the toxic, the frog heart, and the blood pressure methods). Their average variation was 100 per cent. Sowton[7] in the same year assayed 26 British samples by means of the perfused rabbit's heart. Unfortunately Sowton reported her results merely in terms of "weak" or "strong." Goodall,[8] in 1912, assayed 23 tinctures which had been "freshly made by chemists of repute." He used a modified frog method (three minims of the tincture killing a 20-gram frog within four hours). His findings by this method show a departure from the B. P. standard of nearly 50 per cent.

Stewart[9] made a survey of 51 samples purchased at drug stores and reported variations of from 30 to 443 per cent (method of assay not stated).

Symes[10] examined 55 British tinctures, by the twelve-hour frog method, and found that relatively few of his series of tinctures were below the standard strength. They did, however, show variations of from 200 per cent to 300 per cent above standard strength. The findings of Symes are unique, but are probably due to the fact that he used a freshly prepared tincture which did not show time changes.

Kobert,[11] in 1914, standardized a number of tinctures using the twenty-four-hour frog method. He reports variations of over 200 per cent in the official tincture. Roth[12] assayed 13 samples of fat-free tinctures using the one-hour frog method. His report shows variations of 250 per cent, only 3 out of the 13 fat-free tinctures conforming to the U. S. P. standard strength.

Dooley,[13] in 1918, made a survey of tinctures obtained in Syracuse, using the one-hour frog method. His results are interesting in that they show an improvement in the tincture uniformity. Of the official tinctures in his series only two were notably poor, i.e., 27 per cent of normal, the average range for official tinctures being from 85 per cent to 95 per cent of normal. On the other hand, his average of the fat-free samples was 44 per cent of normal. Pratt and Morrison[14] standardized 25 samples by the one-hour frog method and they report that 8, or 32 per cent, met with the requirements of the U. S. P. This work of Pratt and Morrison again shows a tendency toward improvement in tinctures.

Warfield[15] analyzed a group of 9 tinctures, using the thirty-minute frog method, and he concluded that his samples were of weak potency. Lewis and Moffatt[16] analyzed 7 tinctures by the modified cat method. Their findings show that their strongest tincture was approximately two and one-half times as potent as their weakest. Recently Smith and McClosky[17] assayed 7 tinctures, by an intravenous one-hour frog method. Their results show variations up to 175 per cent. This figure is considerably less than that reported by the earlier investigators.

For our method of tincture evaluation, we used the one-hour frog method of Famulener and Lyons,[18] as adopted by the United States Pharmacopoeia, pithing at the end of the hour instead of prior to injection because this mini-

mizes the shock factor. All frogs (Rana pipiens) were obtained from the same locality and were of a fairly uniform size. When received they were held for at least a week in a storage tank in a cool room, the bottom of the tank being covered with running tap water. Before testing they were carefully weighed and then maintained in a suitable tank at 20° C. for one hour prior to the digitalis injection,* and subsequently to the end of the observation period. The alcoholic content of the tinctures assayed was lowered by dilution with 0.7 per cent sodium chloride solution. Ouabain† was used as a standard of comparison.

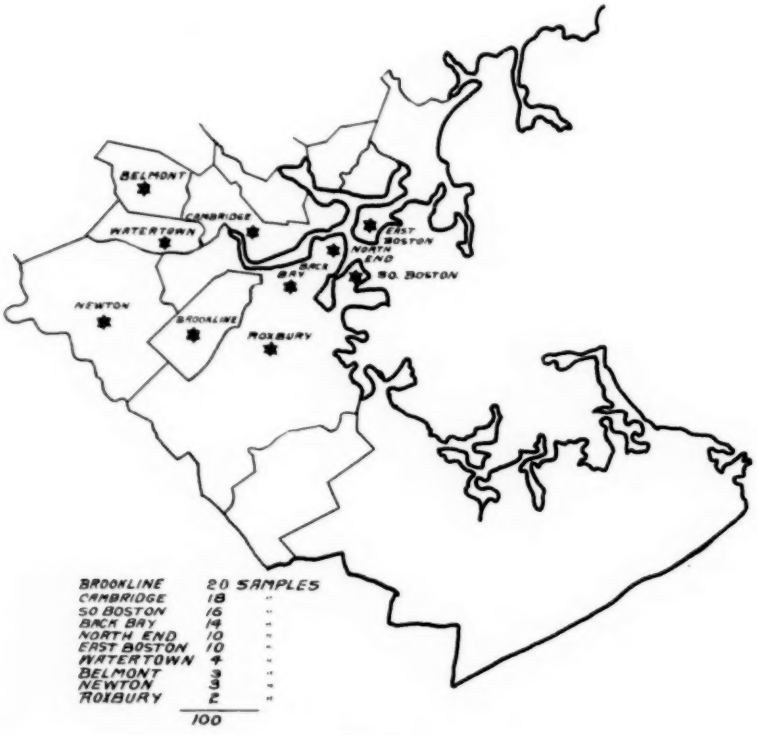

Chart 1.

While it is undoubtedly true that the seasonal variation is a factor, our experiences confirm the findings of Smith and McClosky,[17] viz., "The extreme variations in susceptibility noted heretofore must have been due to irregularities of absorption." We again agree with them that sharpness in end-point is best obtained during the winter months; hence we carried out this survey during this period. Close inspection of the ventral lymph sac at the end of sixty minutes reveals the extent of absorption. In our series all frogs showing evidence of faulty absorption were rejected and repeats were made.

Chart 1 shows the extent of the collection field. Effort was made to collect samples from varying types of drug stores.

*We found the tuberculin syringe more accurate and convenient than the pipette method of injection.

†For this we are indebted to the U. S. Department of Agriculture; Bureau of Chemistry.

TABLE I

A COMPARISON OF THE STRENGTH OF INDEPENDENT DRUG STORE TINCTURES

DISTRICT	NUMBER SAMPLES ASSAYED	PER CENT SAMPLES STANDARD STRENGTH	PER CENT SAMPLES ABOVE STANDARD STRENGTH	PER CENT SAMPLES BELOW STANDARD STRENGTH	PER CENT VARIATIONS IN POTENCY	NUMBER OF FAT-FREE SAMPLES
Brookline	20	40	10	50	60	10
Cambridge	18	11	11	78	120	7
Boston—So. Boston	16	19	0	81	67	4
Boston—Back Bay	14	14	14	72	100	6
Boston—E. Boston	10	20	20	60	80	4
Boston—North End	10	20	30	50	120	1
Watertown	4	50	0	50	50	1
Belmont	3	67	0	33	50	0
Newton	3	0	0	100	14	0
Roxbury	2	0	0	100	14	2

In Table I the variability of 100 samples is shown.* A brief discussion of certain districts follows:

The North End.—Marked variability was found; of the 10 samples which represent this district, half were below the accepted standard, the lowest value being 0.011 gram units.

The Brookline District.—Our survey shows findings here which more closely conform to the U. S. P. requirements than any of the other districts studied. As noted, 20 tinctures were assayed; and of these, 8 fulfilled the Pharmacopoeia requirements. Six caused systolic stand-still with 0.007 gram units; four with 0.008. The remaining two tinctures were above the standard strength, i.e., 0.005 gram units.

Cambridge District.—Eighteen samples were obtained and assayed; 7 of these were fat-free tinctures. The total variation was 120 per cent. Seventy-eight per cent of these samples were below the U. S. P. requirements. Eleven per cent were of correct strength and 11 per cent showed potency above the standard.

Probably the majority of physicians, guided by the advertised U. S. P. tincture standard, continue to administer the tincture in drop units. It appears that in Cambridge as well as elsewhere very few patients receive the intended dose. In many drug stores we were urged to purchase medicine droppers.

South Boston District.—Of the 16 samples from this district, 3 were of correct strength, the remaining 13 being below the official standard, ranging from 0.007 to 0.010.

In this district we were informed by two different pharmacists that alcohol acts as a preservative up to ten years, and that therefore tincture of digitalis cannot show signs of deterioration.

Occasionally a sample would be dispensed by a nonregistered pharmacist. About 25 per cent of the druggists insisted upon name of the purchaser, but only rarely were both name and address taken for the poison book.

*In this table are included the findings pertaining to chain store tinctures and fat-free tinctures which are commented upon separately.

TABLE II

A COMPARISON OF THE ACTIVITY OF CHAIN DRUG STORE TINCTURES

SAMPLE NO.	LOCALITY	GRAM UNITS FOR S. S. S.	FAT-FREE
17	Boston—So. Boston	0.006	–
50	Boston—North End	0.006	–
77	Brookline	0.006	–
78	Brookline	0.006	+
90	Brookline	0.006	–
91	Brookline	0.007	+
68	Cambridge	0.008	–
5	Boston—Back Bay	0.008	–
64	Cambridge	0.009	+
67	Cambridge	0.009	+

Analysis of the potency of these tinctures shows that 50 per cent were of U. S. P. standard strength and 50 per cent were below the Pharmacopoeia requirements, a total variation of 50 per cent. Expressed in gram units the variations extend from 0.006 to 0.009.

While these findings do not show the extreme range of potency that was obtained by assay of independent store tinctures, this series does, however, show the expected variations in tincture strength in samples from a central source but of varying age. Variation will continue until stock bottle methods are abolished and the value of the time factor appreciated. Evaluated in terms of potency, these compare favorably with the independent store tinctures, and the cost per ounce was modest and uniform.

The general tendency in Boston is to give the former official tincture when tincture of digitalis is asked for, but occasionally the druggist will supply the fat-free preparation on his own initiative. Of course, we are quick to realize that the conscientious druggist will fulfill prescription requirements.

In Table III the assay is grouped according to manufacturer. Here is shown in detail the variations that have been previously discussed. Included are three groupings, E, K, and L, which require further comment. Group E is composed of five British tinctures, which are dispensed in sealed brown one-ounce bottles. These are labeled, much as are vaccines and sera, with an expiration date. The value of these precautions is shown in the assay.

Group K represents tinctures made by the druggist. Inquiry here yielded information that is interesting. For example, one of these two druggists stated that sometimes he made his own assay, and insisted that his tincture was of correct strength; the other claimed he assayed his tinctures frequently saying that "otherwise one could not be sure of tincture strength." Our assay of these two tinctures shows that both were below par, i.e., 33 per cent and 50 per cent, but when compared with commercial tinctures, they fall into a middle group as regards potency.

The L group represents samples of uncertain origin dispensed from undated stock bottles, and the information given in response to our inquiry was considered unreliable. Regarding the potency of these samples, our assay showed two which represent our lowest findings, i.e., 0.011 gram units.

Of a total of 77 samples from known manufacturers but three tinctures yielded deterioration requiring 0.010 gram units for sytolic stand-still.

TABLE III

MANUFACTURER

SAMPLE NUMBER	DISTRICT	GRAM UNITS FOR S. S. S.
	Manufacturer A	
92	Boston—East Boston	0.005
47	Boston—North End	0.005
76	Brookline	0.005*
8	Boston—Back Bay	0.005*
20	Boston—South Boston	0.006
49	Boston—North End	0.006
74	Brookline	0.006
83	Brookline	0.006*
86	Brookline	0.007*
12	Roxbury	0.007*
26	Boston—South Boston	0.007*
34	Newton	0.007
36	Watertown	0.007
58	Cambridge	0.007*
59	Cambridge	0.007
80	Brookline	0.007*
71	Brookline	0.007*
85	Brookline	0.008
45	Boston—North End	0.008
75	Brookline	0.008*
79	Brookline	0.008
2	Boston—Back Bay	0.009*
41	Watertown	0.009
14	Boston—Back Bay	0.010
	Manufacturer B	
61	Cambridge	0.005
88	Brookline	0.005*
102	Boston—Back Bay	0.005*
31	Belmont	0.006
37	Watertown	0.006
63	Cambridge	0.006*
99	Boston—East Boston	0.006*
73	Brookline	0.006
22	Boston—South Boston	0.007*
24	Boston—South Boston	0.007*
53	Cambridge	0.007*
97	Boston—East Boston	0.007
11	Roxbury	0.008*
89	Brookline	0.008
93	Boston—East Boston	0.008*
94	Boston—East Boston	0.008
96	Boston—East Boston	0.008*
3	Boston—Back Bay	0.009
	Manufacturer C	
17	Boston—South Boston	0.006
50	Boston—North End	0.006
77	Brookline	0.006
78	Brookline	0.006*
90	Brookline	0.006
91	Brookline	0.007*
68	Cambridge	0.008
5	Boston—Back Bay	0.008
67	Cambridge	0.009*
64	Cambridge	0.009*
	Manufacturer D	
66	Cambridge	0.005
95	Boston—East Boston	0.005
18	Boston—South Boston	0.006*

* = Fat-free.

TABLE III—CONT'D

SAMPLE NUMBER	DISTRICT	GRAM UNITS FOR S. S. S.
	Manufacturer D—Cont'd	
25	Boston—South Boston	0.007
52	Cambridge	0.007
65	Cambridge	0.007*
56	Cambridge	0.008
57	Cambridge	0.008
32	Newton	0.008
13	Boston—Back Bay	0.010*
	Manufacturer E	
43	Boston—North End	0.005
62	Cambridge	0.006
87	Brookline	0.006
100	Boston—East Boston	0.006
21	Boston—South Boston	0.007
	Manufacturer F	
44	Boston—North End	0.005*
48	Boston—North End	0.009
51	Boston—North End	0.009
	Manufacturer G	
38	Belmont	0.006
82	Brookline	0.007
	Manufacturer H	
81	Brookline	0.007*
30	Boston—South Boston	0.010
	Manufacturer I	
84	Brookline	0.006
72	Boston—Back Bay	0.008
	Manufacturer J	
27	Boston—South Boston	0.007
	Manufacturer K	
42	Boston—North End	0.008
60	Cambridge	0.009
	Manufacturer L	
7	Boston—Back Bay	0.006
9	Boston—Back Bay	0.006*
35	Watertown	0.006*
4	Boston—Back Bay	0.007
6	Boston—Back Bay	0.007
16	Boston—South Boston	0.007
19	Boston—South Boston	0.007
23	Boston—South Boston	0.007
28	Boston—South Boston	0.007
29	Boston—South Boston	0.007
33	Newton	0.007
70	Boston—Back Bay	0.007
101	Boston—East Boston	0.007*
15	Boston—South Boston	0.008
40	Cambridge	0.008
39	Belmont	0.009
55	Cambridge	0.009
69	Boston—Back Bay	0.009*
98	Boston—East Boston	0.009
46	Boston—North End	0.011
54	Cambridge	0.011*

* = Fat-free.

The range of variability of these assays is from 0.005 to 0.011, i.e., 120 per cent. This we interpret as evidence of improvement in tincture uniformity. To summarize, our study shows that an assay of 100 tinctures gave the following results: 11 were above the accepted standard; 23 were of the U. S. P. standard strength; 66 were below standard strength.

TABLE IV

A COMPARISON OF THE STRENGTH OF FAT-FREE TINCTURES

DISTRICT	NUMBER OF SAMPLES ASSAYED	PER CENT SAMPLES STANDARD STRENGTH	PER CENT SAMPLES ABOVE STANDARD STRENGTH	PER CENT SAMPLES BELOW STANDARD STRENGTH	PER CENT VARIATION IN POTENCY
Brookline	10	20	20	60	60
Cambridge	7	14	0	86	83
Boston—Back Bay	6	17	33	50	100
Boston—South Boston	4	25	0	75	16
Boston—East Boston	4	25	0	75	33
Roxbury	2	0	0	100	14
Boston—North End	1	0	100	0	0
Watertown	1	0	100	0	0

Our series includes 35 samples of fat-free tincture; a number sufficiently large, we believe, to give additional data showing that this type of tincture is not superior to the former United States Pharmacopoeia tincture.

Twenty per cent (7 samples) fulfilled the U. S. P. requirements, 66 per cent (23 samples) were below normal, and 14 per cent (5 samples) assayed above the normal.

CONCLUSIONS

1. While definite variation in potency was found in digitalis tinctures dispensed in Metropolitan Boston, the evidence offered above indicates that these variations are less than that shown by other similar surveys.

2. We believe that further improvement will take place when all manufacturers market their tinctures in sealed ounce bottles giving an expiration date.

3. Eleven per cent of the tinctures assayed were above the U. S. P. requirements. In cases where massive doses are indicated such overstrength is especially dangerous. This percentage cannot be explained by frog seasonal variation, and the importance of accuracy of standardizing should be appreciated by the manufacturer.

4. Our investigation fails to show that fat-free tinctures are superior to the former U. S. P. tincture.

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THE TREATMENT OF SECONDARY ANEMIA*

(Especially With Various Preparations of Iron)

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HOOPER, Robscheit, and Whipple,[1] in 1920, reported experiments tending to show that Blaud's pills were inert in anemia due to hemorrhage in dogs. Following this, the same and other investigators published numerous reports of both experimental and clinical work on the use of iron in the secondary anemias, the results of which have been much at variance. Musser,[2] in 1921, concluded from his work that "the administration of inorganic iron cannot be said to produce any constant alteration in the course of an experimental hemorrhagic anemia." Sonne[3] reported favorable results clinically with inorganic iron, but stated that he obtained no results with organic iron. Whipple and Robscheit-Robbins,[4] in 1925, again reported that iron was ineffective in "short anemias," but that a favorable reaction was obtained in long-continued, severe anemias due to hemorrhage. Janes,[5] reporting his studies of a group of patients suffering from secondary anemia due largely to chronic infections and parasitic diseases, concluded that iron preparations exert practically no beneficial effect in secondary anemia. Williamson and Ets,[6] whose work was based on experimental hemorrhagic anemia in animals

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found that food iron had a very pronounced beneficial effect, while inorganic iron was inert. Bass and Denzer[7] reported favorable results with iron compounds in anemias of infancy, but pointed out the fact that no response was obtained in anemias due to infection.

Advising the use of massive doses of iron in secondary anemia, Goodall[8], in 1926, stated that "medicinal iron is not to be regarded so much a supply of iron as a stimulus to the utilization of iron." Hart, Elvehjem, Waddell, and Herrin,[9] using nutritional anemia in animals as a basis for study, stated that impure iron salts were effective, while highly purified iron compounds were much less effective. Further experiments by Waddell, Steenbock, and Hart[10] resulted in the conclusion that copper, in certain anemias, may play the part hitherto believed to have been effected by iron, inasmuch as iron compounds freed from all traces of copper were ineffective. However, Mitchell and Schmidt[11] introduced evidence to show not only that iron was effective, but that the more soluble, and therefore the more easily available the iron compound, the more pronounced was its action, whether administered as medicinal iron or as food iron. Whipple[12] reported favorable action with both iron and liver in secondary anemia, and stated that the effect of the two substances combined was equal to the sum of the reactions of both. Whipple and Robscheit-Robbins[13] have recently reported that "iron is the most potent metal so far tested," and that the optimum intake of iron exceeds by three times the loss of iron by bleeding (in experimental hemorrhagic anemias).

Minot, Murphy, and Stetson,[14] in 1928, reported having obtained response of the reticulocytes, similar to that obtained in pernicious anemia, in cases of secondary anemia treated with liver. Keefer and Yang[15] stated from their studies, that iron and liver extract were each effective in bringing about increased hemoglobin regeneration in patients with various forms of secondary anemia, and that together, liver extract and iron gave a better reaction than either alone. Later, the same authors[16] also reported studies of the reticulocytes before and after various forms of treatment; in addition, they stated that "the reticulocyte response in post-infection anemias, aplastic anemias, and most chronic post-hemorrhagic anemias is not pronounced."

During the past eighteen months we have studied a number of cases of secondary anemia of various types. We have selected for this report only patients who have had no blood transfusions, and who have been studied for a sufficiently long period of time to determine definitely whether or not, in a given case, the therapeutic agent was effective. All patients were given the regular hospital diet except as will be stated below, so that the diet factor was constant. The types of anemia include (Table I) anemia from a single massive hemorrhage, from repeated small hemorrhages, from infections, from cancer, from lead poisoning, and one case of sickle cell anemia. The therapeutic agents employed included both organic and inorganic iron in solution and in powder (capsule) form, liver (pernicious anemia and Whipple fractions), and combinations of liver extract and iron.

Whenever the clinical condition of the patient would permit, a control period of seven to fourteen days was allowed before treatment, for a study

TABLE I

CASE NO.	AGE	OBSERVATION (WEEKS)	DIAGNOSIS	BEFORE TREATMENT			AFTER ONE MONTH TREATMENT				THERAPEUTIC AGENT		EQUIVALENT IN GM. PER DAY	
				R.B.C.	HG	RETICULOCYTES	R.B.C.	HG	RETICULOCYTES	RETICULOCYTES AT PEAK	IRON	LIVER	METALLIC IRON	FRESH LIVER
Single Massive Hemorrhage														
1	28	12	Ruptured Ectopic Pregnancy	2.54	38.0	114	3.88	67.9		267	Iron Citrate		0.47	
2	33	6	Ruptured Ectopic Pregnancy	3.16	52.0	76	4.41	73.5	104	238	Fe. Amm. Citrate-Brown		0.39	
3	27	4	Ruptured Ectopic Pregnancy	1.45	18.9	205	3.52	52.0	120	462	Iron Citrate		0.47	
4	29	7	Postpartum Hemorrhage	3.07	50.0	84	4.26	65.7		248	Fe. Amm. Citrate-Green			
5	18	4	Ruptured Urethra	2.18	29.7	92	3.25	37.8		176	Iron Citrate	P. A. Fraction Liver	0.097	75.0
6	26	26	Peptic Ulcer	2.05	41.6	250	3.95	61.2		250		P. A. Fraction Liver		600.0
Repeated Hemorrhages														
7	21	4	Abortion	1.65	27.7	122	3.33	47.1	87	220	Iron Citrate		0.24	
8	34	5	Hemorrhoids	3.21	48.5	32	5.12	83.3			Iron Citrate		0.24	
9	57	10	Hemorrhoids	3.81	29.7		6.08	78.1			Fe. Amm. Citrate-Green		0.39	
10	57	4	Hemorrhoids	2.84	19.1	68	5.00	44.0		267	Fe. Amm. Citrate-Green	Whipple Fraction Liver	0.78	1500.0
11	25	4	Uterine Fibroids	2.27	38.1	72	4.01	66.4	179	400	Iron Citrate		0.24	
12	31	7	Uterine Fibroids	1.63	16.8	32	3.41	56.8	34	327	Iron Citrate		0.24	
13	19	6	Uterine Hemorrhage	3.13	26.7	92	4.66	75.3		450	Iron Citrate		0.24	
14	24	4	Postpartum Hemorrhage	2.00	39.5	14	3.97	78.1	101	173	Iron Citrate		0.24	
15	58	4	Epistaxis	1.72	29.7	48	2.95	41.6	53	170	Iron Citrate	P. A. Fraction Liver	0.19	300.0
16	21	9	Peptic Ulcer	3.16	28.1	29	4.92	48.0		374	Iron Citrate	P. A. Fraction Liver	0.19	300.0

TABLE I—CONT'D

CASE NO.	AGE	OBSERVATION (WEEKS)	DIAGNOSIS	BEFORE TREATMENT			AFTER ONE MONTH TREATMENT				THERAPEUTIC AGENT		EQUIVALENT IN GM. PER DAY	
				R.B.C.	HG	RETICULOCYTES	R.B.C.	HG	RETICULOCYTES	RETICULOCYTES AT PEAK	IRON	LIVER	METALLIC IRON	FRESH LIVER
							Carcinoma							
17	44	6	Carcinoma of Uterus	2.03	16.3	115	3.42	26.5	41	229	Fe. Amm. Citrate-Green	Whipple Fraction Liver	0.78	1500.0
18	51	3	Carcinoma of Uterus	2.26	30.0	208	3.28	44.6	190	380	Ferrous Carbonate		0.39	
19	39	4	Carcinoma of Uterus	2.40	26.0	21	1.70	20.8	36	110	Ferrous Carbonate		0.39	
20	40	5	Carcinoma of Cervix	2.99	31.2	75	3.92	31.2	47	128	Fe. Amm. Citrate-Green		0.39	
							Acute, Subacute, and Chronic Febrile Diseases							
21	71	4	Pyelonephritis	2.79	38.9	67	3.58	45.9	82	180	Iron Citrate		0.36	
22	27	5	Septic Abortion	2.16	38.6	19	4.00	73.5	28	149	Iron Citrate		0.24	
23	26	4½	Pelvic Abscess	2.72	27.6	49	3.56	40.1	121	158	Iron Citrate		0.24	
24	18	3½	Postpartum Sepsis	2.51	39.5	78	4.05	54.3		176	Iron Citrate		0.24	
25	42	18	Multiple Fractures with Infection and Hemorrhage	2.87	55.8	103	3.22	61.2	39	208	Iron Citrate		0.24	
26	29	29	Bronchopneumonia	3.69	31.3	118	3.68	44.6	48		Iron Citrate		0.24	
27	4	4	Empyema	3.46	24.0		4.91	52.0			Iron Citrate	P. A. Fraction Liver	0.097	75.0
							Tuberculosis							
28	26	4	Miliary Tuberculosis	2.40	36.8	17	2.74	37.2	44	55	Iron Citrate		0.24	
29	31	5	Pulmonary and Pelvic T. B.	2.23	27.1	67	3.34	29.7	87	157	Fe. Amm. Citrate-Green	Whipple Fraction Liver	0.78	1500.0
							Lead Poisoning							
30	37	100	Plumbism	4.16	35.9	87	5.03	78.1	87	380	Iron Citrate		0.36	
							Sickle Cell Anemia							
31	9	4	Sickle Cell Anemia	1.92	38.3	175	1.87	40.0	271	382	Iron Citrate		0.24	

of the spontaneous fluctuations in the blood and particularly of the reticulocytes. The source of the anemia was corrected, whenever possible, before treatment was instituted; then the control period followed, in order to determine whether any response which occurred later was spontaneous or induced. After treatment was started, frequent, and for the most part, daily, erythrocyte and reticulocyte counts and hemoglobin estimations were made. During the period of study, no drugs reputed to be of value in the treatment of anemia, other than iron or liver extract, were administered.

It may readily be seen that there is little in common between the experimental nutritional anemias studied by Hart, Steenbock, Waddell, Elvehjem, Herrin, and others, and the more common types of secondary anemia in human beings. The experimental type of anemia dealt with by Whipple and his associates, however, closely resembles the severe anemias due to chronic loss of blood in man. There is a strong possibility that these differences may account for the variance in results obtained. However, we have attempted to determine whether patients respond to iron, not only by increased formation of hemoglobin, but also, whether, following the administration of iron, there is evidence of increased activity of the hematopoietic system in the production of cellular elements.

Table I includes data on 31 patients, who have been divided into groups according to the cause of the anemia. Red blood cell counts (R. B. C.) are recorded in millions per c.mm. Hemoglobin (Hg) is expressed in percentage (Newcomer Method). Reticulocytes are recorded in thousands per c.mm. of blood.

In several instances we have observed no response at all following iron administration. This was particularly true in the single case of sickle cell anemia, in the two cases of advanced tuberculosis, and in the last two cases of the carcinoma group (Table I). Each of these five patients received preparations of iron or of liver and iron which were effective in other patients. These cases may therefore be considered failures from the standpoint of iron therapy.

In other instances there was improvement in the hemoglobin and red blood cell count without appreciable reticulocytosis. A part of this group showed greater increase in hemoglobin than in erythrocytes, so that the color index increased (Cases 12 and 27, Table I). At other times, the proportionate increase in erythrocytes was greater than that of the hemoglobin, so that the color index decreased (as in Case 5, Table I). The latter did not occur commonly, however.

Reticulocytosis was observed in several patients. The increase in reticulocytes usually began about four or five days after treatment was started; the peak occurred most often about the seventh or eighth day, after which there was a steady decline in immature cells to normal numbers, with steadily increasing hemoglobin and number of erythrocytes. This reticulocyte response resembled closely that seen in pernicious anemia patients treated with liver extract, but, in general, occurred slightly earlier. In a few instances, a

moderate reticulocytosis was seen which persisted for many days, and which was associated with steadily increasing hemoglobin and number of red blood cells.

The reticulocyte response in the treatment of pernicious anemia with liver extract or hog's stomach is not appreciable when the level of the red blood cells is much above 3.0 million per c.mm., whereas, with secondary anemia, the reticulocyte response has been observed when the level of the red blood cells was above 3.0 million. In one instance (Case 30, Table I) a definite reticulocyte response occurred at a time when the red blood cells were slightly above 4.5 million. It is suggested that the concentration of the hemoglobin may also be an influencing factor in the magnitude of the reticulocyte response in cases of secondary anemia.

The improvement in the general condition of the patients was most marked in those who showed reticulocyte showers. About the time of onset of the reticulocytosis, there was an increase in appetite; several patients complained of not getting enough food. A few of these who were quite undernourished were given high caloric diets with intermediate feedings, after the peak of reticulocytosis had passed. The increased desire for food persisted after the reticulocyte response was completed. In addition to this, there was a rapid return of strength, increased color of the skin and mucosae, and a general feeling of well-being. Symptoms of anemia, such as dim vision and tinnitus, gradually disappeared. These changes occurred also in patients who responded without reticulocytosis but with increased numbers of erythrocytes and percentage of hemoglobin.

Table II includes cases which gave evidence of a hematopoietic response by reticulocytosis, and only cases in which this response was unquestionably due to treatment. Red blood cell counts (R. B. C.) are recorded in millions per c.mm., hemoglobin (Hg) in percentage, and reticulocytes in thousands per cu.mm.

From an analysis of Tables I and II, it is at once evident that the most striking hematopoietic response occurred in the group of patients whose anemia was due to chronic blood loss (Cases 7 to 16 inclusive, Table I). Of the ten cases in this group, six responded to iron or liver and iron treatment with reticulocytosis, a higher percentage of this type of response than occurred in any other group. Furthermore, the number and percentage of reticulocytes in this group was higher than in the other types of secondary anemia. In addition, it may be stated that all of the iron, and liver and iron preparations used in the treatment of these chronic posthemorrhagic anemia patients, were used in other types without similar results. None of the chronic bleeding patients had persistent fever or infections.

The case of lead poisoning (Case 30, Table I) is particularly interesting from the standpoint of the type of response. This man had been observed for several months before treatment, and at no time showed greater than normal numbers of reticulocytes. At the time the iron treatment was started, the color index of the blood was 0.43. At the end of one month, during which time he received the daily amount of iron citrate equivalent to 0.36 gm. metallic iron, his erythrocyte count had returned to normal, the color index

TABLE II

CASE NO.	DIAGNOSIS	BEFORE TREATMENT			AFTER ONE MONTH TREATMENT		GAIN IN ONE MONTH		RETICULOCYTE PEAK		IRON OR LIVER PREPARATIONS USED	EQUIVALENT IN GM. METALLIC IRON OR FRESH LIVER PER DAY
		R.B.C.	HG	RETICULOCYTES	R.B.C.	HG	R.B.C.	HG	THOUSANDS	PER CENT		
1	Ruptured Ectopic Pregnancy	2.54	39.0	114	3.88	67.9	1.34	28.9	267	9	Iron Citrate	0.47
2	Ruptured Ectopic Pregnancy	3.16	52.0	76	4.41	73.5	1.25	21.5	238	7	Fe. Amm. Citrate-Brown	0.39
3	Postpartum Hemorrhage	3.07	50.0	84	4.26	65.7	1.19	15.7	248	7.2	Fe. Amm. Citrate-Green	0.39
4	Abortion, Hemorrhage	1.65	27.7	122	3.33	47.1	1.68	19.4	220	10.7	Iron Citrate	0.24
5	Hemorrhoids	2.84	19.1	68	5.00	44.0	2.16	24.9	267	7.8	Whipple Fraction Liver	1500.0
											Fe. Amm. Citrate-Green	0.78
6	Uterine Fibroids	2.27	38.1	72	4.01	66.4	1.74	28.3	400	16.9	Iron Citrate	0.24
7	Uterine Fibroids	1.63	16.8	32	3.41	56.8	1.78	40.0	327	14.0	Iron Citrate	0.24
8	Uterine Hemorrhage	3.13	26.7	91	4.66	75.3	1.53	48.6	450	10.9	Iron Citrate	0.24
9	Peptic Ulcer	3.16	28.1	29	4.92	48.0	1.76	19.9	374	10.6	P. A. Fraction Liver	300.0
											Iron Citrate	0.19
10	Cancer of Uterus	2.03	16.3	115	3.42	26.5	1.39	10.2	229	8.2	Whipple Fraction Liver	1500.0
											Fe. Amm. Citrate-Green	0.78
11	Lead Poisoning	4.16	35.9	87	5.03	78.1	0.87	42.2	380	8.3	Iron Citrate	0.36
	Average	2.69	31.8	81	4.22	59.0	1.52	27.2	309	10.0		

had increased to 0.59, and the reticulocyte count was normal. During this month, he had increased numbers of reticulocytes for twenty-seven days. The peak of reticulocytes, 8.3 per cent or 380,000, was reached on the ninth day after treatment was started and at a time when the erythrocyte level was 4.58 million per c.mm. There was very marked improvement in his clinical condition, and he was soon able to return to his work.

The dose of both the iron and the liver preparations has varied considerably. The smallest daily dose of metallic iron we have used was 0.24 gm. when iron alone was given; the largest daily dose of metallic iron was 0.78 gm. The smallest daily dose of liver extract was the amount derived from 75 gm. of fresh liver (No. 343 or pernicious anemia fraction). The largest daily dose of liver extract used was the amount derived from 1500 gm. of fresh liver (Whipple fraction). The highest percentage of reticulocytes obtained was (Case 6, Table II) after the administration of iron citrate in daily amounts equivalent to 0.24 gm. of metallic iron. However, from the available data, it cannot be concluded that the combined use of liver and iron is not more effective than iron alone. Indeed, the only patient in the cancer group who had a reticulocyte response due to treatment received large doses of both iron and liver extract (Case 10, Table II).

The equivalent in metallic iron of one Blaud's Pill (U.S.P.) is about 0.03 gm. It is therefore quite evident that the dose of iron salts employed was in every case rather large (equal to eight Blaud's Pills in those instances where the smallest doses of iron salts were administered). However, in conjunction with an extract of liver, the smallest dose was the equivalent of 0.09 gm. of metallic iron daily, or a dose equal to about three Blaud's Pills. The maximum dose of iron salts used was equivalent in metallic iron to 0.78 gm., or a dose equal to about 26 Blaud's Pills per day. Despite the fact that relatively large doses of the iron salts were used, we have observed no toxic symptoms from overdosage.

All of the effective iron preparations used in the treatment of this series of patients were readily soluble, and therefore easily available for absorption in the body. We have used commercial preparations of the iron salts such as may be purchased in any pharmacy, and which, therefore, may have contained small amounts of other elements, as copper, manganese, etc. The content of iron, copper, etc., in the diet has not been determined, but this factor is not of great importance as the patients who have not responded received the same diet as those who did respond.

Riecker[17] has pointed out that a sharp distinction must be drawn between the type of response characterized merely by increased hemoglobin formation and that characterized by cellular regeneration of the blood elements. Both types of response have occurred in this group of patients. Insofar as can be determined at present, there is no plausible explanation for the presence of reticulocytosis in some patients after iron therapy, and the absence in others, when all have increased hemoglobin formation and increased numbers of red blood cells. It is also difficult to determine the explanation of the failure of iron therapy reported by some observers. It may be that solubility, and therefore availability, and the massive doses of

iron are factors influencing the response, which probably accounts for a certain number of the failures reported in the literature.

In a few instances the hematopoietic response was retarded or interrupted by intercurrent infections, by intoxications, or by fever. In other patients, who had long-continued infections with fever, no response was seen. Zerfas and Smithburn[18] have shown that fever and intoxication may inhibit the hematopoietic response in pernicious anemia patients treated with liver extract. The mechanism of this inhibitory influence is obscure, but it may be that infectious processes, acute or chronic febrile diseases, or systemic intoxications are not so much concerned with actual destruction of the elements in the circulating blood as with a deleterious effect on the hematopoietic system.

In the groups of anemias due to acute and chronic hemorrhage (Cases 1 to 16, Table I), it might appear that the reticulocytosis was the result of hemorrhage. However, observations during the control period gave definite information in regard to this point on which this factor of possible error can be eliminated. Furthermore, it may be stated that several of these patients continued to have hemorrhage during treatment. It is reasonable to believe that hemorrhage may cause reticulocytosis, but it does not follow that the patient who has a spontaneous reticulocytosis following hemorrhage, *and who continues to bleed* will show increased formation of hemoglobin and increased numbers of erythrocytes.

In studying the effect of any therapeutic agent in a group of patients with secondary anemia, many problems are confronted which cannot be accurately controlled as in a strictly scientific animal experiment. The studies included in this report are, in some respects, therefore, not comparable to the reports on experimental anemias to which we have previously referred. However, the results to be expected from methods similar to these in the treatment of patients with secondary anemias are often of undoubted benefit and are occasionally spectacular.

SUMMARY

1. Soluble iron salts are of definite value in the treatment of certain cases of secondary anemia when administered in sufficiently large doses.

2. Patients with carcinoma, or with acute or chronic infections attended by fever, do not respond readily, if at all, to iron therapy.

3. Fever, infection, or intoxication may be responsible for temporary or complete inhibition of the hematopoietic response during treatment with iron salts, liver extract, or both. This may possibly be due to some deleterious effect on the hematopoietic system.

4. The results of iron therapy are most marked when the amount of iron in the body has been depleted, as by long continued anemia from loss of blood over a prolonged period of time.

5. The administration of iron salts may produce an increase in the concentration of hemoglobin and the number of red blood cells, either with or without a reticulocyte response. Usually, the reticulocyte response is observed when the initial level of the red blood cells is 3.0 million per c.mm. or

below. A reticulocyte response has occurred, however, when the level of the red blood cells was above 4.0 million per c.mm., and may have been related to the very low concentration of hemaglobin in the blood.

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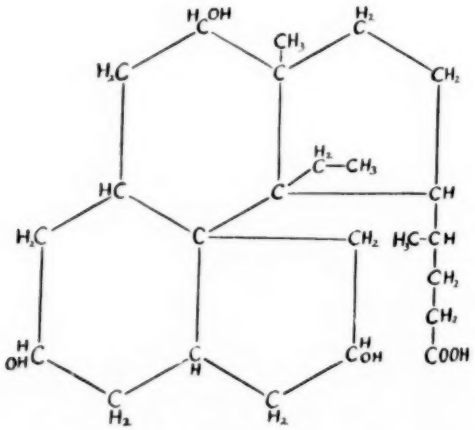
SODIUM DEHYDROCHOLATE*

Its Specific Effect on Pneumococci, II

By Edwin E. Ziegler, M.D., Northport, N. Y.

IN PREVIOUS work of the author[1] bile salts were injected intravenously in the treatment for pneumonia. The salts used were a mixture of sodium glycocholate and sodium taurocholate. The success which attended their use was marred by the fact that they produced injury to the veins for some distance above the point of injection. It was suggested that other salts or esters of the bile acids, or other bile acids, be tried in the treatment for pneumonia. In following out this thought an investigation of sodium dehydrocholate was undertaken and its effect on pneumococci in vitro was studied.

It is known that cholic acid is the active part of the molecule of the various bile salts causing the specific lytic effect on pneumococci. The rational formula of cholic acid is $C_{24}H_{40}O_5 + H_2O$. According to the work of Wieland,[2] Borsche and Frank[3] on the constitution of the bile acids, the structural formula attributed to cholic acid is shown.



Cholic Acid

Cholic acid is oxidized to dehydrocholic acid, the alcohol groups of the cholic acid changing to carbonyl groups. Dehydrocholic acid occurs as a fine colorless crystalline powder having the rational formula, $C_{20}H_{33}(CO)_3COOH$. Its crystals are needle-shaped and have a melting point of 233 to 235° C. The sodium salt dissolves readily in water, less readily in alcohol. It oxidizes

*From the U. S. Veterans Hospital.
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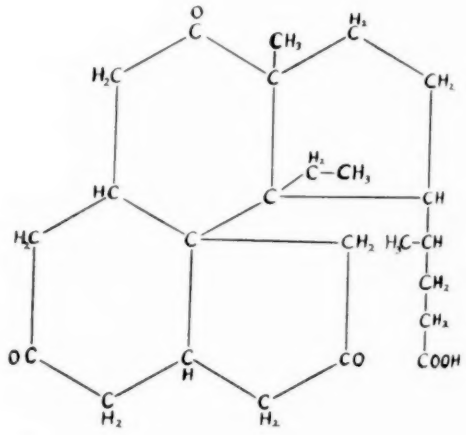
slowly in the air. Its solution in water has a light yellowish-brown or straw color, is weakly alkaline and has a bitter taste. Its structural formula is shown.



Dehydrocholic Acid

It was desirable to determine the effect of sodium dehydrocholate on pneumococci as this salt has been found to be the least toxic of the bile salts and because it can be given intravenously in quite large doses and in convenient concentrations without injury.

Neubauer,[4] in an experiment on the choleretic effect of sodium dehydrocholate, injected a patient having a biliary fistula, with two grams of this salt in a 20 per cent concentration without injury. The same worker has performed numerous other experiments with the bile salts showing that sodium dehydrocholate was the least toxic.

On account of the hemolytic properties of the salts of the bile acids, they were regarded as relatively toxic substances. However, Neubauer showed that sodium dehydrocholate holds a prominent position among the bile salts in being practically nontoxic on intravenous administration. He conducted experiments with bile salts to determine their hemolytic properties, their action on isolated frog's hearts, and their effect on animals. He found sodium dehydrocholate to be practically nontoxic and nonhemolytic. Compared with cholic acid, dehydrocholic acid was about 200 times less hemolytic. Neubauer found also that hemolysis was much less if the bile salts were dissolved in serum. He also demonstrated that the isolated temporaria heart (after Straub) required a 2 per cent solution of sodium dehydrocholate to stop systole in ten minutes; a 1:100 concentration causing only a reversible decrease of the systole, and a 1:200 concentration had practically no effect. In contrast to this, sodium desoxycholate stopped the beating of the heart after one minute in a 1:1,800 concentration. Neubauer also injected a dog in the ear vein with large doses of sodium dehydrocholate without ill effect; there was no certain slowing of the pulse and no hemoglobinuria. Repeated

injections of very large doses caused diarrhea. Gillert[5] determined in his experiments the fatal doses of bile salts to rabbits as follows (Table I):

TABLE I

	GRAMS PER KILOGRAM	
	MAXIMUM NONFATAL DOSE	LETHAL DOSE
Sodium dehydrocholate	1.0	1.1
Sodium tuarocholate	0.10	0.11
Sodium glycocholate	0.08	0.09
Sodium apocholate	0.07	0.09
Sodium cholate	0.05	0.05
Sodium desoxycholate	0.01	0.015

From this it appears that sodium dehydrocholate is about ten times less toxic than sodium taurocholate and glycocholate which I injected intravenously in man, in previous experiments.[1] The lethal dose of sodium dehydrocholate in rabbits is unusually high. This dosage cannot be directly calculated on a human basis but serves as a rough guide for the estimation of dosage in man.

Adlersberg and Taubenhaus[6] tested the effect of sodium dehydrocholate on the blood pressure of eighteen patients. Six patients showed no change in blood pressure. Twelve patients, who had some degree of hypertension, showed a decrease in blood pressure from 12 to 30 mm. Hg.

Using the same general methods as in previous work the hemolytic effect of sodium dehydrocholate and of sodium taurocholate were determined, and thus compared. Table II shows the hemolytic effect of sodium taurocholate

TABLE II

HEMOLYTIC EFFECT OF SODIUM TAUROCHOLATE

TIME	PERCENTAGE CONCENTRATION										
	5	4.5	4	3.5	3	2.5	2	1.5	1	0.5	C
10 minutes	4+	3+	3+	3+	2+	2+	+	+	0	0	0
15 minutes	4+	3+	3+	3+	2+	2+	2+	+	+	0	0
45 minutes	4+	3+	3+	3+	2+	2+	2+	2+	+	+	0
75 minutes	4+	4+	3+	3+	3+	3+	3+	3+	2+	2+	0
3 hours	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	0

Each tube contained 0.5 c.c. suspension blood cells 1:10 plus 0.5 c.c. of sodium taurocholate. In tube C 0.5 c.c. of 0.85 per cent NaCl plus 0.5 c.c. of blood cells. C, control; 0, no hemolysis; +, slight hemolysis; 4+, complete hemolysis; 2+ and 3+, stages of hemolysis.

TABLE III

HEMOLYTIC EFFECT OF SODIUM DEHYDROCHOLATE

TIME	PERCENTAGE CONCENTRATION										
	5	4.5	4	3.5	3	2.5	2	1.5	1	0.5	C
45 minutes	0+	0+	0+	0	0	0	0	0	0	0	0
75 minutes	0+	0+	0+	0+	0+	0+	0	0	0	0	0
3 hours	+	0+	0+	0+	0+	0+	0+	0	0	0	0
20 hours	4+	3+	3+	2+	2+	+	+	0+	0	0	0

Each tube contained 0.5 c.c. suspension blood cells 1:10 plus 0.5 c.c. of sodium dehydrocholate. In tube C 0.5 c.c. of 0.85 per cent NaCl plus 0.5 c.c. of blood cells. C, control; 0, no hemolysis; 0+, trace hemolysis; +, slight hemolysis; 4+ complete hemolysis; 2+ and 3+, stages of hemolysis.

and Table III that of sodium dehydrocholate. The experiments represented in these tables were conducted at room temperature as follows: The respective salts were prepared in 0.5 c.c. amounts in ten test tubes ranging in concentration from 1 to 10 per cent. The eleventh tube was a control containing 0.5 c.c. of 0.85 per cent NaCl solution. To each tube of both series was added 0.5 c.c. of a 1:10 suspension of blood cells. The suspension of blood cells was prepared by centrifuging 10 c.c. of defibrinated human blood, pouring off the supernatant serum and then diluting the suspension of cells to 100 c.c. with 0.85 per cent NaCl in water. The blood cells were not washed and of course a minimum amount of serum remained in the preparation. The final concentrations of the dilution series of each salt was of course, only half the amount after the blood cell suspension was added, that is, from ½ to 5 per cent.

This series of experiments shows that under conditions, in which a 5 per cent solution of sodium taurocholate produces complete hemolysis in ten minutes, it takes a 5 per cent solution of sodium dehydrocholate twenty hours to produce the hemolysis. This indicates that sodium taurocholate is about 120 times as hemolytic as sodium dehydrocholate and gives the latter salt a great advantage over sodium taurocholate for intravenous therapy.

The crucial test of sodium dehydrocholate from the standpoint of this work was to determine its effect on pneumococci in vitro and to compare the results with the lysis of pneumococci by sodium taurocholate. This was done by performing lysis experiments as follows: Dilution series of test tubes were prepared containing 0.5 c.c. of the bile salt solutions. A series was prepared for each type of pneumococcus and for each bile salt. To each tube was then added 0.5 c.c. of the appropriate type of pneumococcus suspension. The experiments were conducted at room temperature. Readings were made at intervals as indicated in the tables. The final concentrations of the bile salts ranged from 1:25,000 to 1:20.

Suspensions of pneumococci, Types I, II, and III were obtained by growing them in the peritoneal cavities of young rabbits. The animals were given chloroform before death and the peritoneal cavities washed out with normal salt solution. Pus cells were largely removed from the suspensions by a short period of centrifugation. This method gives the most satisfactory suspensions. Broth cultures or other suspensions containing culture material are objectionable. Mice can be used to grow the pneumococci. They were used in previous work but young rabbits were selected as large amounts of suspension were needed.

The experiments were performed with the three types of pneumococci[7] and with the two bile salts, making a total of six experiments. The six experiments were each repeated three times using pneumococcus suspensions of different concentrations. These different suspensions gave slightly different readings and the Tables IV and V represent a mean of the results. In heavy suspensions of pneumococci, a slight amount of lysis is not as readily detected as in lighter suspensions. As the results with each type of pneumococcus were so nearly the same, they were consolidated into one table.

The important result from this work is the discovery that sodium dehydrocholate dissolves pneumococci, of live and virulent types, in the same manner as sodium taurocholate. The two bile salts, in the same concentrations, and with the same pneumococcus suspensions produce the same amount of lysis in practically the same time.

TABLE IV

LYSIS OF PNEUMOCOCCI (TYPES I, II, AND III) BY SODIUM TAUROCHOLATE

TIME	PERCENTAGE CONCENTRATION									
	5	2	1	0.5	0.1	0.05	0.01	0.005	0.004	C
10 minutes	2+	+	0+	0+	0+	0+	0+	0	0	0
45 minutes	3+	3+	2+	2+	+	+	0+	0	0	0
2 hours	4+	4+	4+	4+	3+	3+	3+	2+	2+	0

Each tube contained 0.5 c.c. pneumococcus suspension plus 0.5 c.c. of sodium taurocholate solution, concentrations as shown. C, control, 0.5 c.c. p. suspension plus 0.5 c.c. of 0.85 per cent NaCl solution. 0, no lysis; 0+, slight lysis; 4+, complete lysis; +, 2+, and 3+, stages of lysis.

TABLE V

LYSIS OF PNEUMOCOCCI (TYPES I, II, AND III) BY SODIUM DEHYDROCHOLATE

TIME	PERCENTAGE CONCENTRATION									
	5	2	1	0.5	0.1	0.05	0.01	0.005	0.004	C
10 minutes	2+	+	0+	0+	0+	0+	0+	0	0	0
45 minutes	3+	3+	3+	2+	+	+	0+	0	0	0
2 hours	4+	4+	4+	4+	3+	3+	3+	2+	2+	0

Each tube contained 0.5 c.c. pneumococcus suspension plus 0.5 c.c. of sodium dehydrocholate solution, concentrations as shown. C, control, 0.5 c.c. p. suspension plus 0.5 c.c. of 0.85 per cent NaCl solution. 0, no lysis; 0+, slight lysis; 4+, complete lysis; +, 2+, and 3+, stages of lysis.

DISCUSSION

A relationship of the bile salts to the immunity mechanism of pneumococcus infections has been demonstrated by me,[1] by Elton[8] and by Castallanos.[9] Ross[10] has shown that bile salt dissolved pneumococci when filtered through a porcelain filter and fed to animals, induces a very high degree of immunity to subsequent lethal doses of pneumococci. He also showed that this immunity is not demonstrable by serologic antibody tests. The immunity mechanism in pneumonia seems to be on a simpler chemical level.

Neufeld's phenomenon,[11] i.e., the lysis of pneumococci by bile, has been found to take place with very dilute solutions of certain bile salts. It was found by the work represented in this paper that sodium dehydrocholate dissolves pneumococci in the same time and in the same dilute solutions as other bile salts previously used. It was also shown that sodium dehydrocholate is practically nonhemolytic as compared to sodium taurocholate and glycocholate. Other workers, previously mentioned, have shown that sodium dehydrocholate is practically nontoxic when given in large doses to patients intravenously. The only unfavorable symptom reported from the intravenous administration of sodium dehydrocholate in large amounts is a diarrhea. This is produced by the increased output of bile induced by the bile salts.

In previous work, formerly referred to, the author used a solution of a 50 per cent mixture of sodium taurocholate and glycocholate intravenously

in the treatment for pneumonia. The dose of the salts ranged from 1 to 8 gm. with no toxic symptoms except local injury to the veins.

Sodium dehydrocholate can be obtained in a chemically pure, sterile condition; and can be given intravenously in a 20 per cent solution.

In view of these findings it is proposed to abandon other bile salts for the time and to try sodium dehydrocholate intravenously as a treatment for pneumococcus pneumonia and for pneumococcus infections in general.

CONCLUSIONS

1. Sodium dehydrocholate is the least toxic of the bile salts and is practically nonhemolytic; being at least 120 times less hemolytic than sodium taurocholate and glycocholate.

2. Sodium dehydrocholate dissolves pneumococci in the same time and in the same dilute concentrations as the other bile salts.

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VITAL FUNCTION STUDIES*

VIII. Weight Calculation in Pregnancy

By Allan Winter Rowe, Ph.D., Boston, Mass.

SOME ten years ago the author began a study of certain phases of the metabolism in normal pregnancy as a basis for a later comprehensive investigation of the toxemias incident to this condition, a line of approach that had already yielded concrete results in another field.[1] Many of the measurements undertaken demonstrate a real significance only if presented as relative values or as changes, using convenient and easily ascertained biometric magnitudes as bases of reference. A case in point is the so-called basal metabolic rate, all of the prediction criteria involving some expression of the body weight in the formula used.

While in normal pregnancy the patient can be weighed directly, in many of the toxic cases the physical condition precludes direct measurement. The present report deals with a series of correlations with formulas based upon the direct observation of normal women for the calculation of this magnitude. Single measurements on isolated cases fall far short of the significance attaching to continuous studies on the same individual, and while the adoption of the latter procedure added materially to the labor involved and to the difficulties of performance, the advantages were felt to be more than compensatory, therefore, the work has been carried out on this basis. Normal, healthy individuals were selected initially and only those retained who main-

Table I

Physical Measurements

DATUM			AMOUNT	DATUM			AMOUNT
Number of Cases			31	Weight	High	(kg.)	87.0
				1 week a.p.	Low	(kg.)	49.8
Age	High	(yr.)	28		Av.	(kg.)	*66.8*
	Low	(yr.)	14				
	Av.	(yr.)	*19*	Chest	High	(cm.)	99
				1 week a.p.	Low	(cm.)	73
Height	High	(cm.)	167.4		Av.	(cm.)	*81.8*
	Low	(cm.)	151.8				
	Av.	(cm.)	*160.2*	Hips	High	(cm.)	114
				1 week a.p.	Low	(cm.)	89
Sitting Height	High	(cm.)	91.5		Av.	(cm.)	*99*
	Low	(cm.)	80.6				
	Av.	(cm.)	*85.7*	*Average Weekly Increase*			
				Weight			*+0.50* kg.
S. H. Index			*0.534*	Chest			*+0.50* cm.
				Hips			*+0.25* cm.

*From the Evans Memorial, Boston.
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Presented in part at the Annual Meeting of the American Physiological Society, Chicago, Illinois, April, 1930.

tained this condition throughout the period of study. For the purposes of the general programme of investigation several series of patients drawn from widely different sources were studied. Group "C," composed of the inmates of a nursing home for unmarried mothers, furnished the data here considered.

Certain descriptive details can be presented most compactly in tabular form (Table I).

The large number of young mothers depends upon the source from which the patients were drawn. Comparisons with the data of older patients serving as controls, however, demonstrates the representative character of the physical measurements of the group.

Weights were taken at a uniform hour in the early morning, after emptying the bladder, with the patient nude and in a postabsorptive state (i.e., at least twelve hours after the last meal). No allowance could be made for variations in the amount of the intestinal contents. A standard precise, platform type of scale was used, the accuracy of which was checked at intervals.

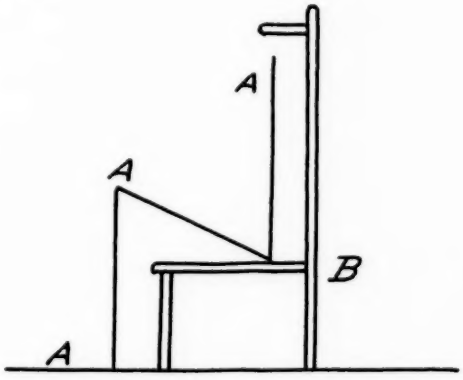

Fig. 1.—*A A*, Body line. *B*, apparatus.

Circumferential measurements were made with steel tapes directly over the skin. The chest measure was made directly below the breasts, the waist at the level of the umbilicus, and the hips at the point of greatest circumference.

Standing heights were measured with the patient erect and using calipers of standard design. The sitting height, or as Dreyer[2] erroneously designates it, "trunk height," could not be secured in the manner described by him, as many of the patients found the posture difficult if not impossible to assume. To meet this contingency, a standard measuring rod with sliding caliper was firmly fixed to a small stool, ten inches in height and twelve inches square, and the patient was placed on this with the buttocks firmly pressed against the standard and the lower leg in a vertical position. This puts the gluteals under substantially the same tension as the Dreyer posture and yielded results of an excellent reproducibility. Comparative measurements with a control group, using both postures, failed to disclose significant differences. All measurements were made in duplicate and checked independently.

While the standing and sitting heights are practically constant throughout pregnancy, the weight, chest, and hip measurements show a progressive increase. Average values for the coefficients of change appear in Table I. Final measurements one week antepartum are given as it is probable that a loss of weight supervenes in the last days before delivery. The chest and hip values also would be affected by this circumstance. Waist measures are omitted from the table as the individual woman shows fluctuations that render this observation meaningless. That this should be the case might well be anticipated from the parts of the body involved.

The major purpose of this portion of the study was to secure data from which body weight could be calculated for those patients whose physical condition did not permit of a direct measurement.

The Dreyer prediction formulas for weight correlated with either sitting height or chest girth are not directly applicable to this purpose. In the case of the first, the predicted weight is for an individual of normal configuration and can take no cognizance of departure from this standard. Its original purpose was to define a normal standard with which observed weights could be compared. As the author elsewhere has shown,[3] the "sitting height" can be secured for a recumbent subject by measuring the distance from the perineum to the vertex with a pair of special calipers and, for the female, adding 1.1 cm. to the value thus obtained.

The chest girth, on the other hand, is a measurement directly influenced by the nutritional level of the individual, a fact that, even for its original purpose, renders it of service only in those cases where the configuration is a normal one. Both obesity and emaciation lead to extrapolations of absurd and misleading magnitudes, as has already been pointed out.[3] For example, a woman 150 cm. tall and weighing 129.5 kg. was 5 per cent below the weight predicted from her chest girth, while by the sitting height comparison she was 179 per cent overweight, the latter a more accurate expression of the true condition.

As noted above, the waist measure is worthless because of the daily variability, the recorded magnitudes precluding any reliable definition.

In the paper already cited,[3] it has been shown that the hip girth taken at the point of maximum circumference can be correlated with body weight and the latter predicted with a definite degree of accuracy by means of the following formula.

Female

$$\text{Weight in grams} = 1.387 \times \sqrt[0.429]{\text{Hip Girth in cm.}}$$

One other initial factor presents itself for consideration. As the thesis is to determine a method for the calculation of the body weight of the recumbent individual, the influence of this position on the two circumferential measurements, hips and chest, must be ascertained.

Taking the data of the thirty-one cases in this series, it is found that in the antepartum period the hip measurement standing shows an average value of 0.7 cm. more than that in the recumbent state. Further, 74 per cent of the

patients show a difference of 1 cm. or less, and the remainder are not materially superior to the upper limit of variation. The same figures postpartum are respectively 0.4 cm. and 81 per cent.

Similar measurements on the chest show deviations of about the same magnitude but opposite sign. The average standing chest measure antepartum is 0.8 cm. less, and that taken postpartum 0.5 cm. less than the complementary observations in a recumbent position. The percentages of those differing by not more than 1 cm. are respectively 66 and 73. When one considers the tensional factors in the dependent tissues, the direction of these changes are those to be anticipated.

Comparison of observed weights with those predicted by the several formulas must take due cognizance of the state of pregnancy; one measure only, the sitting height, will not be materially affected by the weight increase and so deviations from it should be progressive. The several comparisons are grouped in Table II. The averages are drawn directly from the assembly of the original data and not by computation from the coefficients given in Table I. This allows the individual variations to find expression. The postpartum figures are included as controls.

TABLE II

AVERAGE WEIGHT DEVIATION FROM PREDICTION

TIME (WEEKS)	HIP	TRUNK	CHEST
24-21	+2%	− 1%	0%
20-17	+4%	+ 5%	− 4%
16-13	+4%	+ 9%	− 9%
12- 9	+5%	+ 8%	− 9%
8- 5	+6%	+10%	−13%
4	+7%	+10%	−12%
3	+6%	+12%	−13%
2	+7%	+14%	−11%
1	+6%	+16%	−15%
	Delivery		
3	−4%	−1%	−8%
4	−2%	−1%	−4%
5- 8	0%	−2%	−2%
9-12	+4%	−1%	−2%
13-16	+4%	+2%	+3%
Over 16	+3%	+5%	−5%

In the antepartum period, the hip prediction follows the actual observation most closely, but shows a slight upward tendency, that of the sitting height exhibits the steady upward progress of the added weight, and the chest values illustrate once more the complete dependence of this standard upon wholly normal body configuration. Even the uterus and its contents cannot compensate for the influence of the fat deposits on the chest.

Postpartum, with definite loss of weight and restoration of relatively normal bodily contour, all of these of the formulas yield values that are in close accord with the observed weights.

These points are even more strikingly illustrated in individual case protocols, and the details of a like comparison applied to a very obese and to a definitely underweight patient are given in Table II-a.

TABLE II-A

EFFECT OF OVER- AND UNDERWEIGHT

TIME (WEEKS)	WEIGHT	HIP	TRUNK	CHEST
		Case C-24		
17	75.8	+11%	+22%	−16%
15	77.0	+16%	+24%	−38%
13	78.2	+15%	+25%	−36%
11	79.1	+16%	+27%	−31%
9	80.9	+19%	+30%	−35%
7	81.6	+17%	+31%	−30%
5	81.2	+15%	+30%	−33%
3	82.6	+16%	+33%	−47%
		Delivery		
3	74.0	+ 2%	+19%	−33%
14	83.6	+ 3%	+34%	−30%
21	88.1	+ 6%	+42%	−35%
30	88.4	+ 7%	+43%	−40%
		Case C-26		
4	54.2	+ 3%	− 3%	+ 1%
3	57.2	+ 1%	+ 2%	+ 2%
		Delivery		
8	50.3	− 2%	−11%	+14%
10	50.9	− 1%	−10%	+22%
14	50.4	− 4%	−11%	+20%
25	47.3	− 4%	−16%	+24%

In the case of the overweight patient, even the hip formula gives values significantly divergent from the truth during the antepartum period, while the residual overweight, after delivery, affects both trunk and chest values in the same sense as in the earlier period.

With the thin patient, the hip formula is satisfactory, while the other two show significant deviations postpartum with reversal of sign from the similar data of the obese patient. It must be repeated that the hip formula was developed to permit of weight calculation, while the other two were intended to define normal criteria as a basis for the mathematical expression of deviations.

The object of this present study is to develop a method for the calculation of the weight of the bedridden pregnant patient. While the hip formula offers a satisfactory approach for the average individual even with the unusual weight addition produced by pregnancy, its deviation with the markedly obese pregnant patient may exceed allowable limits. On the other hand, the same agency that influences the sitting height comparison in one direction affects the chest correlation in the opposite sense. Inspection of the data of the last two tables shows that the mean of the trunk and chest deviations gives values that are really close to the weight actually observed. That this should be so with the average values is not surprising, but the results of a similar manipulation in the extreme cases is nearly as good, and in the obese case (C-24) definitely superior to the hip formula.

Before going farther with this phase of the discussion, it is desirable to ascertain the influence of the weight error on the basal rate prediction. The two standard formulas, those of Harris-Benedict[4] and of Aub-duBois,[5] use body weight, in the first case directly, in the second as a means of predicting

body area which is then used in the final computation. Taking the extremes in this series, it is found in round numbers that the weights vary from 45 to 90 kg., the heights from 147 to 175 cm., and the ages from fourteen to twenty-eight years. Calculating the several basal rates that the permutations of the basic data allow, it can easily be shown that an error of ±10 per cent in the weight will affect values with Harris-Benedict formula by from 4 per cent to 5 per cent, and with the Aub-duBois from 5 per cent to 6 per cent. Adopting arbitrarily a ±10 per cent error in the weight calculation as the maximum of permissible variation, all of the observations in the group (250 in number) can be analyzed from the standpoint of the relative accuracy of prediction. The first consideration, namely, the relative accuracy of the hip formula as opposed to the mean of the sitting height and chest predictions, is given in Table II-b.

TABLE II-B

ANALYSIS OF RELATIVE ACCURACY OF WEIGHT PREDICTION BY HIP AND BY SITTING HEIGHT-CHEST MEAN

NUMBER	HIP FORMULA IS BETTER	EQUAL	POORER
Antepartum 163	29%	26%	45%
Postpartum 87	45%	21%	34%

Obviously the hip formula offers the greater dependability in the postpartum period, while the combined sitting height-chest figure is preferable during pregnancy. As this latter is the period of concern for the present purpose of this study, further analysis of the individual data is informative. With the hip formula, 82 per cent of the values fall inside the arbitrary limit of ±10 per cent, while the combined formula shows the better record of 93 per cent. The average deviation of the hip values irrespective of sign is 4.5 per cent, for the combined formula, 4.1 per cent. Of the deviations outside the adopted limit, the average with the hip is 13 per cent, and 14 per cent with the combined formula.

One last modification may be dealt with briefly. Practically all of the hip deviations are positive, in other words, the observed weight is greater than that calcualted from the hip formula. If 5 per cent be added arbitrarily to the value as calculated, one hundred and fifty-one of the values fall inside of the limit of ±10 per cent, with an average variation of 4.2 per cent. Further, the average for the twelve measurements lying outside (nine of which came from two obese patients) is 12 per cent. In other words, there is an almost complete parity in the reliability of the modified hip formula and that of the combined sitting height-chest calculation. Both methods permit of an approximate computation of the body weight that falls within allowable limits in over 90 per cent of the series of observations. This further, in a group of measured weights showing extreme variations from the normal, as calculated by Dreyer's sitting height formula, of from 22 per cent below to 62 per cent above prediction.

Recognizing the limitations intrinsic to any such form of calculation, and equally, the large errors likely to be introduced in cases presenting the selective distribution of adipose such as the girdle obesity of the pituitary and torso overdevelopment of the ovarian failure, the formulas permit of a first approximation of a biometric magnitude that is essential to the just interpretation of a variety of metabolic data.

CONCLUSION AND SUMMARY

The contents of this paper may be briefly summarized as follows:

1. Groups of pregnant women, maintained under divergent domiciliary conditions, have been studied continuously during both the ante-and postpartum periods.

2. Increments in weight and other simple physical measurements antepartum, and decrements after delivery have been recorded.

3. As earlier noted, the sitting height in the recumbent female case can be approximated by adding 1.1 cm. to the distance between the perineum and vertex, as measured with special calipers.

4. The hip circumference in a recumbent position during pregnancy averages 0.7 cm. *less* than in a standing posture; the difference averages 0.4 cm. after delivery.

5. Similar comparisons show that the recumbent chest measure averages 0.8 cm. *more* than that of a standing pregnant subject; the difference is 0.5 cm. after confinement.

6. The actual weight of the recumbent pregnant woman may be calculated with reasonable accuracy in the majority of cases either.

   (a) by adding 5 per cent to the prediction of the hip formula, or
   (b) computing the algebraic mean of the predictions respectively from Dreyer's sitting height ("trunk") and chest formulas.

The author takes much pleasure in acknowledging his obligations to the staffs of the Robinson Memorial, the Talitha Cumi Home, and the House of Mercy, and to the several groups of patients for their help and cooperation; also to Dorothy E. Gallivan and Helen Matthews, by whom these measurements were made.

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STUDIES ON ANESTHESIA:

II. Certain Effects of Low Oxygen Concentrations*†

By Reginald Stuart Hunt, Ph.D., Boston, Mass.

DURING the last three decades the estimation of the so-called basal metabolic rate and the comparison of the observed level with those normal values based upon various prediction formulas[1, 2] have assumed very real diagnostic significance. Particularly during the last ten years the clinical applications of this procedure have been investigated, and an extensive literature testifies to the widespread interest in the respiratory metabolism in both health and disease.

As the term is used it implies the energy requirement of the individual measured under carefully defined conventions, namely, in a postabsorptive state and in a condition of complete physical and mental relaxation. The first of these criteria is easy of accomplishment; the second offers a grave source of error in many determinations, a fact that has escaped the recognition of the observer in a regrettably large number of recorded measurements. As all of these subversive influences operate to increase the observed rate, irrespective of the method of determination used, records are actually of minima and the true rate may be appreciably below that reported from the experimental data. With a large part of the public, repetitions of the test serve as an educational agent and permit a progressively closer approximation of the true basal value. There remains, however, a certain and not negligible number who mentally or physically are unable to cooperate to the point of complete compliance with the requirements. In this category fall the very little children and those of older years who have been encouraged in vociferous self-expression—an unhappily large group in these United States—the highly neurotic, the psychotic, and those individuals with partial loss of movement control as in the Parkinson syndrome.

To secure authoritative observations on these highly important groups, it was suggested by Rowe[4] that the use of anesthetic or hypnotic agents might produce the necessary relaxation and degree of cooperation. That the drug effect would influence the oxygen consumption seemed probable and this pointed to the necessity of measuring such effects in the hope that conversion coefficients might be experimentally determined.

The present investigation and several other studies now under way are the outcome of this suggestion. Scattered in the literature are individual reports of the influence of certain drugs, but there is lacking any complete

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study of the various agents suitable for present purposes. With the more common anesthetic agents, the several methods of use and administration show differences which are potentially significant to the immediate thesis. Among other agents are the gas anesthetics, such as nitrous oxide and ethylene commonly administered in so high a concentration that the complementary oxygen which completes the mixture is present in an amount much below that of the atmospheric content. Benedict,[5] among others, has shown that the oxygen consumption is not sensibly increased with concentrations of oxygen superior to the air even up to the practically pure gas. It has been recognized, on the other hand, that a lowered oxygen intake produces definite changes in the oxygen requirement of the individual. The existing data lack some clarity in outline; some of them are possibly influenced by extraneous factors, such as the lowered barometric pressures of Schneider's[6] comprehensive investigations, and others are apparently contradictory in sense as well as in amount. An initial step in the complete plan of study was the expres-

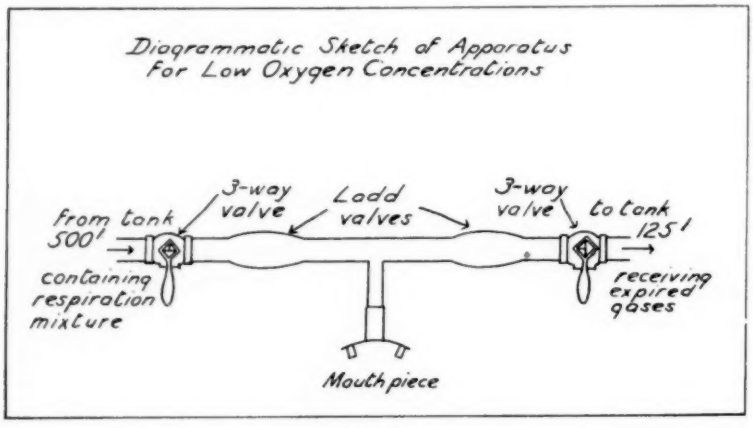

Fig. 1.

sion of this influence in quantitative terms, and this present and wholly preliminary report deals with certain results which thus far have been secured.

The subjects for the study were demonstrably healthy young men and women, volunteers drawn from the student body of Boston University School of Medicine and from the technical staff of the Evans Memorial. The individual subject reported to the laboratory at 8:30 A.M. in a post-absorptive state (i.e., after a fast of not less than twelve hours), and on arrival was given a rest period of not less than thirty minutes in a recumbent position, adequately covered and with loosened clothing. When repeated pulse readings gave evidence of basal relaxation, a measurement of the oxygen requirement was made with the Benedict Collins[7] respiration unit with internal blower and using practically pure oxygen. Throughout this and the subsequent portion of each experiment, frequent observations were made of pulse and respiration rates. Body temperatures were frequently measured orally with standardized mercury thermometers* and a number of

*This procedure is far from ideal but represents a compromise necessary to secure full cooperation with a varied group of volunteer subjects.

blood pressure records were taken with a mercury column instrument provided with a broad soft cuff and using an auscultatory tambour.

The apparatus for measurements with reduced oxygen concentrations consisted of a large 500 liter Tissot spirometer of standard design for the respiration mixture and a similar instrument of smaller capacity (125 liter) to receive the expired gases. Large bore rubber tubes with a pair of Ladd valves suitably placed connected the two spirometers with the standard rubber mouthpiece through which the subject was made a part of the system. Standard nose clips were used to close the nostrils. The general plan is shown in the accompanying diagrammatic sketch (Fig. 1).

A preliminary run of ten minutes was made to familiarize the subject with the apparatus and to fill the connections of the collecting spirometer with a gas mixture representative of the output of the experiment. The by-pass was then opened and the collection chamber emptied, during which operation the subject continued to breathe the respiration mixture; the apparatus was then closed and a fifteen-minute experimental period run, timed with an accurate stop watch. Subsequent observations duplicated this procedure in all details. The respiration mixture and the expired gases were analyzed for CO_2 and O_2 in duplicate with the well-known Haldane portable apparatus. This instrument was checked daily by one or more analyses of atmospheric air. All gas volumes and measurements were reduced to standard conditions for final expression.

The respiration mixtures were prepared by diluting atmospheric air contained in the large spirometer with known amounts of nitrogen. The gases were thoroughly mixed by an inside fan and then allowed to stand overnight. Analyses of composition were made at the time of use.

A few of the pertinent data relating to the physical factors of the group of subjects are given in Table I.

TABLE I

PHYSICAL MEASUREMENTS

DATUM			MALE	FEMALE
Age	High	(yr.)	34	33
	Low	(yr.)	20	22
	Average	(yr.)	**23**	**25**
Height	High	(cm.)	190.5	165.4
	Low	(cm.)	161.7	153.0
	Average	(cm.)	**173.9**	**160.1**
Weight	High	(kg.)	91.1	80.5
	Low	(kg.)	50.3	46.0
	Average	(kg.)	**67.9**	**56.2**
Area	High	(sq. m.)	2.10	1.87
	Low	(sq. m.)	1.58	1.44
	Average	(sq. m.)	**1.79**	**1.59**
Total number			**55**	**10**

In the male group, it is evident that there was a fairly representative scatter. The women were too few in number and the measurements with them too limited in scope to yield authoritative values. The sexes must be considered separately in many of the observations recorded, as intrinsic sex dif-

ferences are well known, and the absolute values of any mixed group would be influenced by the percentile composition. The respiratory quotients, to be considered later, constitute an exception to this general rule, and for this reason certain of the female records are utilized in this portion of the report.

The first influence studied was that of lowered oxygen in the respired gases upon the actual oxygen consumption. As the subjects varied somewhat widely in height and weight, it was necessary to adopt a standard biometric unit to rationalize the comparison. As the DuBois formula for surface area[2] is based upon these two quantities, the square meter of area was adopted as the basic criterion of reference. The data are given in Table II.

TABLE II

OXYGEN CONSUMPTION (MALE GROUP)

PER CENT OF OXYGEN IN MIXTURE	MINUTE VOLUME	OXYGEN PER MINUTE PER SQUARE METER OF AREA			
		HIGH OXYGEN B-C APPARATUS	REDUCED O_2 PER CENT	DIFFERENCE C.C.	DIFFERENCE PER CENT
20.94	3.45	134	137	+ 3	+ 2.2
17.71	2.75	133	138	+ 5	+ 3.8
15.98	3.65	133	136	+ 3	+ 2.3
14.25	3.77	139	133	− 6	− 4.3
13.30	3.83	133	152	+19	+14.3
12.12	3.22	132	138	+ 6	+ 4.5
10.60	4.49	133	131	− 2	− 1.5
9.63	4.59	134	123	−11	− 8.2

In the first column are given the average oxygen percentages for each of the groups of observations. The third column records the results of the standardizing measurements made with approximately pure oxygen in the Benedict Collins apparatus on the individuals composing each group. The uniformity of these figures is an earnest of the uniformity in the composition of each subdivision. Only the figure in the 14 per cent series departs by more than one per cent from the average. In the 21 per cent (air) group, the consumption is 3 per cent higher than where high oxygen and the Benedict machine is used. This could be interpreted as measuring the additional work of overcoming valve and spirometer resistance and the figures at 18 per cent, 16 per cent, and 12 per cent would severally bear out this explanation. Unfortunately, the average at 14 per cent is wholly inconsistent with such an interpretation, especially as it is followed by the anomalous figure at 13 per cent which will be discussed later. Frankly, it seems more reasonable to regard the differences, at the higher oxygen concentrations at least, as signifying no more than the summation of individual variations recorded in the single group.

The results with 13 per cent oxygen constitute a definite and disturbing exception to the reasonable uniformity of the earlier records. Analysis of the original data shows two subjects who exhibited very large increases when shifted to the lower oxygen tension, but even with the omission of these data—an arbitrary procedure for which no warrant exists—the average of the remainder still stands disproportionately high.

A. Loewy, L. Loewy, and L. Zuntz[9] have recorded increases of respectively 6.3 per cent, 20.1 per cent, and 16.5 per cent (average +14.6 per cent)

at an altitude of 12,000 feet, or about 13 per cent oxygen; however, in a pneumatic cabinet at the same barometric pressure (448 mm.) the same observers showed an increase in oxygen consumption of only +3.5 per cent, a figure in fair conformity with many of the deviations of this series. It may be that this concentration represents a critical point of readjustment in the respiratory mechanism; the more reasonable conclusion is that the number of observations in the group is too few to absorb certain individual anomalies and give an average really representative of normal performance. The matter is now under investigation.

While the difference at 10.6 per cent suggests the initiation of a compensatory mechanism, the arithmetical magnitude is too small to warrant such an interpretation. At 9.6 per cent, however, the figures leave no doubt that an equilibrium is being established at a lower level. This tendency has also been recorded by Schneider[6(f)] using lowered barometric pressure as a means of decreasing oxygen concentrations. The latter found, however, that this lowering effect was transitory and that during continued exposure to this pressure for several hours, there was a gradual rise in the oxyygen requirement which ultimately equalled or even exceeded that shown by the individual with 21 per cent of the gas. For the basic purpose of this study, however, long continued exposure does not come into consideration; that there is an initial drop of the order of about 10 per cent is suggested by these data.

Analyses of expired gases for both CO_2 and O_2 give opportunity to determine the changes in the apparent respiratory quotient. The term "apparent" is used selectively, as the over ventilation produced by lowered oxygen concentrations induces the "pumping out" effect on CO_2 that has been so frequently recorded by others. The respiratory quotients recorded in this study do no more than give arithmetical form to this effect. Incidentally, they bear directly on some of the problems incident to gas or vapor anesthesia. The data are collected in Table III.

TABLE III

INFLUENCE OF LOWERED OXYGEN UPON THE OBSERVED RESPIRATORY QUOTIENT
(Total Group)

PER CENT OF OXYGEN IN MIXTURE	RESPIRATORY QUOTIENT	
	OBSERVED	DIFF. FROM AIR
20.94	0.83	±0
17.70	0.84	+.01
15.66	0.88	+.05
14.49	0.87	+.04
13.38	0.89	+.06
12.12	0.89	+.06
11.41	0.91	+.08
10.46	1.02	+.19
9.63	1.16	+.33

Patently, the influence manifests itself with only slight decrease in the available oxygen and exhibits a progress that becomes very significant in amount as one approaches the lower concentrations. The changing quotients

as represented graphically define a well marked curvilinear relationship as shown in the accompanying plot. (Fig. 2.)

The equation from the curve may be of service in the resolution of some of the problems connected with inhalation anesthesia. The tendency to become asymptotic is manifest at 11 per cent and becomes pronounced at concentrations inferior to 10 per cent.

One objective of this investigation was the influence of lowered oxygen tension on the observed basal rate. Ordinarily these are—or should be—calculated from the oxygen consumption and when closed circuit devices, such as the Benedict Collins apparatus, are used, with the assumption that the respiratory quotient remains at 0.82. That this is a permissible convention is too well established to require argument. Reference to Table II shows that there is but little change in the oxygen consumption with the exception

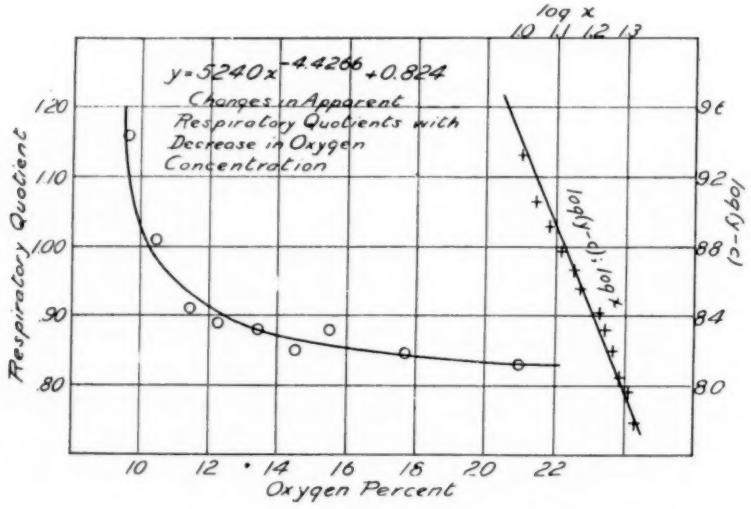

Fig. 2.

of the uncertain figure at 13 per cent and the definitely lower value at 9.6 per cent—both of which have already been discussed. To calculate basal rates from calorific values for oxygen based upon such artefactual R.Q. values as those given in Table III would serve only to mask the real values of the respiratory exchange. Assuming then, a calorific value for oxygen, equivalent to the conventional R.Q. of 0.82, and calculating the basal rates from the oxygen consumption with the two types of apparatus, a series of data is produced calculated to disclose the influence of the changing oxygen tensions. The results are collected in Table IV.

Comparisons have been made with the Harris-Benedict predictions only, for the sake of simplicity. The usual practice of this laboratory is to report the algebraic mean of this prediction and that derived from the Aub-DuBois generalization. As is to be expected, considering the source of the basic datum, the indications here follow those of Table II. The anomalous value

TABLE IV

BASAL METABOLIC RATE DEVIATION (MALE GROUP) COMPARED WITH HARRIS-BENEDICT PREDICTION

PER CENT OF OXYGEN IN MIXTURE	WITH HIGH OXYGEN PER CENT*	WITH REDUCED OXYGEN PER CENT*	DIFFERENCE
20.94	−3%	± 0	+ 3%
17.71	−5%	− 2%	+ 3%
15.98	−3%	+ 2%	+ 5%
14.25	+1%	− 3%	− 4%
13.30	−3%	+10%	+13%
12.12	±0%	+ 4%	+ 4%
10.60	−2%	− 3%	− 1%
9.63	−1%	−10%	− 9%

*Calculated from oxygen consumption with R. Q. = 0.82. See text.

at 13 per cent repeats in this series and the depression engendered by the lowest oxygen concentration again manifests itself. It seems reasonable to conclude, as a first approximation only, that concentrations of oxygen such as occur in gas anesthesias are sufficient in themselves to lower the basal rate from 5 to 10 per cent.

As this laboratory has frequently emphasized, no basal rate report can be regarded as complete which fails to include the records of pulse, respiration, body temperature, and blood pressure taken at the time of the determination. The data for the first three are gathered in Table V.

TABLE V

EFFECT OF LOWERED OXYGEN ON PULSE AND RESPIRATION (Male Group)

PER CENT OF OXYGEN IN MIXTURE	TEMPERATURE	PULSE			RESPIRATION		
		HIGH O_2	RED. O_2	DIFF.	HIGH O_2	RED. O_2	DIFF.
20.94	98.0	72	72	± 0	14	14	±0
17.71	97.9	74	75	+ 1	14	12	−2
15.98	98.0	62	63	+ 1	17	17	±0
14.25	98.2	75	77	+ 2	16	17	+1
13.30	98.4	75	81	+ 6	16	15	−1
12.12	97.9	69	84	+15	14	15	+1
10.60	98.1	71	90	+19	14	15	+1
9.63	98.0	61	85	+24	16	19	+3

But few comments are necessary. A slight and progressive upward tendency to the pulse rate manifests itself with slight decrements of the oxygen tension and assumes significant values between 13 per cent and 12 per cent. The complementary figures for the respiration rate show an increase of real magnitude only at concentrations below 10 per cent. A review of the minute volumes (Table II) will show, however, that an increase in respiratory effort develops somewhat earlier but expresses itself in an increase in the amplitude of the single respiration.

Similar data for the blood pressure records are collected in Table VI.

As might be anticipated, the systolic blood pressure shows an upward tendency with even moderate decrements of available oxygen, which is fol-

TABLE VI

EFFECT OF LOWERED OXYGEN ON BLOOD PRESSURE
(Male Group)

PER CENT OF OXYGEN IN MIXTURE	WITH HIGH OXYGEN		WITH LOW OXYGEN		DIFFERENCE	
	SYS.	DIAS.	SYS.	DIAS.	SYS.	DIAS.
20.94	117	72	117	72	± 0	±0
17.71	128	86	128	85	± 0	−1
15.98	119	67	112	65	− 7	−2
14.25	126	76	128	75	+ 2	−1
13.30	120	70	126	70	+ 6	±0
12.12	119	76	123	72	+ 4	−4
10.60	118	70	125	72	+ 7	+2
9.63	112	66	123	70	+11	+4

lowed by a slight rise in the diastolic pressure at the two lowest levels. Schneider and Truesdell[6(c)] found a drop in diastolic pressure, but their experiments were conducted in an entirely different manner (progressive O_2 decrement by rebreathing and removal of CO_2).

In addition to the findings reported elsewhere it was noted that, practically without exception, the subjects in the 9.6 per cent oxygen group exhibited anoxemia and dyspnea and complained of a frontal headache or dizziness or both. These conditions disappeared in every instance after a few minutes' exposure to normal oxygen concentration.

SUMMARY

1. A preliminary report is made on a series of experiments with healthy individuals to determine the influence on the respiratory exchange of lowered O_2 concentrations at standard barometric pressure.

2. At concentrations below 10 per cent, the subjects gave evidence of physiologic disturbance and increased respiratory effort. In spite of this, there was an actual lowering of the oxygen consumption of the order of approximately 10 per cent.

3. Basal rates calculated from the preceding data show a similar decline below prediction.

4. At slight decrements in O_2 concentration below that of the atmosphere, a "pumping out" effect on expired carbon dioxide is manifest, and this progresses to a degree that produces respiratory quotients superior to 1.00 at the lower levels. The equation for these changes has been computed.

5. At low O_2 concentrations, the pulse rate shows a notable increase; the respiration also increases somewhat but in less marked degree than does the minute volume; the systolic blood pressure rises significantly and the diastolic levels show an upward trend.

In conclusion, I wish to extend my thanks to Dr. Allan Winter Rowe for advice and criticism during the conduct of this study; also to Miss Mary A. McManus who has assisted in many of the measurements and analyses; finally, to the subjects of the experiments for their willing sacrifice of time and convenience.

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LABORATORY METHODS

A PICRIC ACID BLOOD-SUGAR METHOD AFTER ZINC PRECIPITATION*

By Victor C. Myers, Ph.D., Sc.D., and Clinton W. Root, M.A.,
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FOR some years the picric acid method for the determination of blood sugar has been advantageously employed in clinical and laboratory studies. This procedure, as developed by Lewis and Benedict[1] and later modified by Myers and Bailey,[2] has certain advantages; it is convenient as well as "foolproof" and does not require delicate nor complicated reagents. The method, however, has largely fallen into disuse because the blood-sugar values obtained are slightly higher than those given by other methods. The color development is based on the fact that in alkaline solution picric acid is readily reduced by glucose to the reddish-brown sodium salt of picramic acid. Picric acid precipitation, like the tungstic acid used by Folin-Wu, gives values which are approximately too high by about 20 mg. or over. Somogyi[3, 4] has demonstrated the unusual efficiency of zinc as a precipitant not only for blood proteins, but also for those nonglucose reducing substances that explain the high results obtained by the ordinary blood-sugar methods. From Somogyi's work it seemed that the usefulness of zinc might also be applied to the picric acid procedure, and in addition throw some light upon the high values obtained with the ordinary technic. A new procedure has been developed that retains most of the advantages of the old picric acid method and considerably increases its accuracy. This new technic has been compared with the Folin-Wu[5] and the new Benedict[6] methods, and in addition a study has been made of the colorimetric efficiency of picric acid, its proportionality of color and relation to dilution.

EXPERIMENTAL PROCEDURE

Reagents.—In precipitating the proteins two reagents are used. Reagent I contains 50 gm. of $ZnSO_4.7H_2O$ dissolved in water and 125 c.c. N H_2SO_4, the whole being made up to one liter. Reagent II is 1.5 N NaOH. The two reagents are so related that 15 c.c. of Reagent I will exactly neutralize to the phenolphthalein end-point 3.95 c.c. to 4.05 c.c. of Reagent II. The glucose standard is a 0.02 per cent solution of pure glucose in saturated picric acid. Saturated sodium carbonate (22 per cent) is also used in the determinations.

Method.—In a 15 c.c. cylindrical centrifuge tube are placed 3 c.c. of water and 2 c.c. of well-mixed oxalated blood. These are mixed and allowed to

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stand until hemolysis takes place. A stirring rod is used to mix the contents of the centrifuge tube. To the laked blood are added 4 c.c. of Reagent I, the whole becoming much darker in color. This mixture is stirred carefully with the glass rod and to it is added slowly drop by drop with stirring 1 c.c. of Reagent II. The contents of the tube are allowed to stand three minutes and become a distinct milk-chocolate color. The tube is centrifuged for several minutes, when the zinc hydroxide with the proteins and other precipitated substances will be packed very closely to the bottom. In a small funnel is placed about three-quarters or a gram of picric acid on a 3 or 4 cm. filter paper. The clear fluid on top of the precipitated proteins is slowly poured, drop by drop, on the picric acid on the filter paper. This saturates sufficiently the clear filtrate with picric acid and at the same time catches any particles that are not thrown to the bottom by the centrifuge.

Three c.c. of the filtrate are pipetted into a tall narrow test tube, 12 × 200 mm. (Myers and Bailey sugar tube) graduated at 3, 4, 10, 15 and 20 c.c. One c.c. of saturated (22 per cent) sodium carbonate is added and the tube heated in a beaker of boiling water for fifteen to twenty minutes. Simultaneously 3 c.c. of the 0.02 per cent solution of glucose standard in saturated picric acid, which keeps permanently, is treated similarly with sodium carbonate in a sugar tube and heated at the same time as the unknown. This serves as the standard. Under the action of heat and alkali the yellow sodium picrate is converted to the reddish-brown sodium picramate in proportion to the amount of the sugar present. The solutions are now cooled to room temperature either by allowing the tubes to stand or by placing them in a beaker of cold water. The solution in the standard tube is made up to exactly 10 c.c. with water. The contents of the unknown tube are now diluted to 10, 15, or 20 c.c. or some other definite volume which approximates the color of the standard. A little time must elapse after diluting before accurate color comparisons can be made.

Occasionally in certain blood samples a yellowish-white coagulum comes out on heating. This, however, presents no difficulty, for after diluting, a moment in the centrifuge leaves the colored solution free from turbidity caused by the coagulum. It does not seem to occur in fresh normal bloods, but pathologic bloods occasionally present this difficulty. If a coagulum does appear, it must be removed before any colorimetric readings are made.

The standard may conveniently be set at the 15 mm. mark on the colorimeter. Since 3 c.c. of the blood filtrate employed are equivalent to 0.6 c.c. of blood and 3 c.c. of the standard solution contains 0.6 mg. of glucose, the proportions are the same as though 100 c.c. of blood and 0.1 gm. of glucose were employed.

Calculations.—For the calculation the following formula may be used:

$$\frac{S \times D \times 0.1}{R \times 10} = \text{mg. per cent blood sugar.}$$

S represents the depth of the standard 15 mm. and D the dilution of the unknown, 0.1 the strength of the standard in grams of glucose calculated on the basis of 100 c.c. of blood, R the reading of the unknown and 10 the dilution of the standard.

EXPERIMENTAL DATA

The Myers and Bailey modification of the Lewis-Benedict method has been compared with the Folin-Wu and Benedict copper (II) method on human blood under a number of different conditions. Determinations were made in duplicate, the figures recorded representing average results.

TABLE I

COMPARISON OF THE PICRATE BLOOD-SUGAR METHOD WITHOUT AND WITH ZINC PRECIPITATION

BLOOD SAMPLE NO.	SUGAR BY MYERS-BAILEY METHOD		DIFFERENCE
	PICRIC ACID PRECIPITATION	ZINC-PICRIC ACID PRECIPITATION	
	mg.	mg.	mg.
1	122	104	18
2	109	90	19
3	142	125	17
4	129	108	21
5	99	79	20
6	98	77	21
7	74	53	21
8	64	44	20
9	72	59	13
10	116	99	17
11	84	64	20
12	132	109	23
13	103	85	18
14	106	88	18
15	212	186	26
16	196	173	23
17	181	159	22
18	156	134	22
19	176	153	23
20	156	135	21
21	141	121	20
22	107	84	23
23	78	61	17
24	112	87	25
25	216	197	19
26	142	121	20
27	205	180	25
28	130	106	24
Average	131	110	21

Data are recorded in Table I on 28 different blood specimens in which the Myers-Bailey technic was applied to the usual picric acid filtrate, and after preliminary precipitation of the blood with zinc. It will be noted that on an average the values by the modified method were lower by 21 mg. In accord with the observations of Somogyi[4] it was noted that the blood samples having a large cell volume gave a greater lowering of the blood sugar when zinc was used as the protein precipitant. This probably accounts in a large measure for the variations observed in the nonsugar-reducing substances.

Comparison was then made with the Folin-Wu method on a series of 6 specimens. As shown in Table II, the Folin-Wu method gave an average of 123 mg., in comparison with 105 mg. by the modified picric acid method, a difference of 18 mg. Data recently presented[6, 7] would seem to indicate that the results by the original Folin-Wu method were too high by about 22 mg., a greater difference than that recorded in Table II. On this account it seemed

desirable to compare the two methods on the same filtrate, both with and without zinc precipitation. Such comparisons are given in Tables III and IV. It will be noted that the two methods differ by about the same amount in

TABLE II

COMPARISON OF THE BLOOD-SUGAR VALUES OBTAINED WITH THE FOLIN-WU AND ZINC-PICRIC ACID METHOD

BLOOD SAMPLE NO.	FOLIN-WU METHOD	ZINC-PICRIC ACID METHOD	DIFFERENCE
	mg.	mg.	mg.
1	109	83	26
2	100	83	17
3	75	61	14
4	107	87	20
5	208	196	12
6	137	121	16
Average	123	105	18

TABLE III

COMPARISON OF THE BLOOD-SUGAR FIGURES OBTAINED WITH THE ORIGINAL MYERS-BAILEY AND FOLIN-WU METHODS

BLOOD SAMPLE NO.	MYERS-BAILEY	FOLIN-WU	DIFFERENCE
	mg.	mg.	mg.
1	107	100	7
2	78	75	3
3	112	107	5
4	216	208	8
5	142	137	5
Average	131	125	6

TABLE IV

COMPARISON OF THE BLOOD-SUGAR FIGURES OBTAINED WITH THE MYERS-BAILEY AND FOLIN-WU METHODS ON ZINC FILTRATES

BLOOD SAMPLE NO.	MYERS-BAILEY	FOLIN-WU	DIFFERENCE
	mg.	mg.	mg.
1	181	179	2
2	106	103	3
3	211	198	13
4	214	205	9
5	144	134	10
6	155	147	9
7	58	54	4
Average	152	145	7

both cases, namely 6 and 7 mg. The fact that the Folin-Wu method gives slightly lower results on the zinc filtrate than the picric acid method would suggest that there may still be left in the zinc filtrate a nonglucose-reducing substance which affects the picric acid but not the copper of the Folin-Wu reagent.

As a further basis of comparison simultaneous analyses were made employing the modified picric acid method and the new Benedict copper method,[6] which appears to give results very close to the true blood sugar on tungstic acid filtrates. Eight samples of blood were used, the first four being

on normal subjects and the last four on hospital patients. The data are presented in Table V, and it will be noted that the agreement between the two methods is very close, when one considers the great difference in the methods. The Benedict copper method averaged about 1 mg. lower, although this difference is well within the limits of error of the methods.

TABLE V

COMPARISON OF THE ZINC-PICRIC ACID METHOD FOR BLOOD SUGAR WITH THE BENEDICT COPPER METHOD (II)

SAMPLE	BENEDICT COPPER METHOD (II)	ZINC-PICRIC ACID METHOD	DIFFERENCE
	mg.	mg.	mg.
1	84	86	2
2	70	73	3
3	81	82	1
4	73	74	1
5	127	130	3
6	66	67	1
7	110	110	0
8	120	118	2

As a further check on the accuracy of the method known amounts of glucose, 50, 100, and 150 mg. were added to 3 separate samples of normal human blood and sugar estimations made. An inspection of the data presented in Table VI shows that recovery error averaged about 2 per cent, an error no greater than might be expected from the matching of colors in the determination.

TABLE VI

RECOVERY OF ADDED GLUCOSE WITH ZINC-PICRATE METHOD

BLOOD SAMPLE NO.	GLUCOSE ADDED	GLUCOSE PRESENT	GLUCOSE FOUND	ERROR
	mg.	mg.	mg.	per cent
1	0	–	93	–
	50	143	141	1.4
	100	193	197	2.1
	150	243	241	0.8
2	0	–	89	–
	50	139	138	0.7
	100	189	194	2.6
	150	239	233	2.5
3	0	–	92	–
	50	142	141	0.7
	100	192	199	3.6
	150	242	248	2.5
Average				1.9

In order to test out the reliability of the color proportionality of the sodium picramate formed in the reaction, other glucose standards equivalent to varying amounts of glucose from 50 to 1000 mg. were compared with the 100 mg. glucose standard (0.02 per cent glucose in saturated picric acid). In one case water was added to bring the unknown to about the same color intensity as the standard, while in the other case the dilution was kept constant at 10 c.c. From an inspection of the data given in Table VII, it will be ob-

TABLE VII

ACCURACY OF PICRATE METHOD WITH SINGLE STANDARD (EQUIVALENT TO 100 MG.) OVER RANGE 50 TO 1000 MG.

ACTUAL VALUE OF UNKNOWN	FOUND AFTER DILUTION	FOUND WITHOUT DILUTION
mg.	mg.	mg.
50	49	49
75	74	74
100	99	99
150	149	148
200	199	202
300	291	300
500	495	503
700	721	717
1000	1010	940

served that the errors, with one exception, are no greater than those of colorimetric reading, that is, 1 to 3 per cent. The extreme error of 6 per cent was made in attempting to read 1000 mg. of glucose at a dilution of 10 c.c. It has not been our custom, however, to make color comparisons until the unknowns have been diluted to approximately the same color intensity as the standard. The above data indicate that the sodium picrate formation is directly proportional to the glucose present, and that dilution introduces very little, if any, error in the determination.

Data similar to those recorded in Tables VI and VII had been obtained at the time of the publication of the paper by Myers and Bailey,[2] although at that time it was merely stated that glucose added to blood could be quantitatively recovered. The data are presented at this time for the reason that they appear to indicate that with the picrate method one has little to fear in using water as the diluent when the unknown is much stronger than the standard. This would not seem to be the case with the copper methods, where serious errors may be introduced, unless one has a series of standards or employs some special diluting fluid.

DISCUSSION

In 1925 Benedict[8] made the following comment: "To many it may appear unjustifiable to include the picric acid methods here, but I venture to assert that although many thousands of analyses have been made by the picric acid methods, it is doubtful whether a clinician has ever been misled in his interpretation of a diabetic case by the figures obtained by these analyses when properly carried out." Although the method has been largely superseded by various copper methods, a few laboratories have continued to use it owing to its great simplicity.

What have been the main objections to the picric acid method of blood-sugar estimation? The chief criticism has been, of course, that the results were too high. Two other disadvantages of the method are that it requires 2 c.c. of blood instead of the 1 c.c. employed by most of the copper methods, while the reddish yellow color is more difficult to match than the blue of the Folin-Wu and other copper methods.

It has never been denied that the results with the picric acid method were a little too high, but simply claimed that they were comparable. According to the data now available the results obtained on whole blood with such methods as the Folin-Wu have been about 22 mg. too high. The fact that picrate method gave results a few mg. higher would not appear to have made any great difference. With the zinc precipitation employed in the analyses here reported, the results appear to approach those for the true glucose content, thus removing this objection. When one is working with venous blood, it makes little difference whether 1 or 2 c.c. of blood are required for a determination. It is quite true that the reddish yellow of the picrate is harder to match than the blue of the Folin-Wu and other copper method, and one might well have an error of 2 per cent in matching the reddish-yellow color in contrast to 1 per cent with the blue color. With the colorimetric copper methods, however, one may introduce serious errors if high blood sugars are found and certain precautions are not taken, such as the use of several standards or a special diluting fluid. With the picrate method this is not necessary since, as pointed out above, there is a direct proportionality between the amount of glucose and the sodium picrate formation over a very wide range. One standard is adequate whether the unknown contains 50 or 1,000 mg. of glucose. Furthermore, delicate, complicated and expensive reagents are not employed in the method. For these reasons the picrate method of determining blood sugar would appear to be the most nearly "foolproof" of any of the blood-sugar methods. With the use of the zinc precipitation described in the present paper the method gives blood-sugar values which approach those for the true blood sugar. There would appear to be no good reason why this modified method should not be used for almost any blood-sugar determination where 2 c.c. of blood are available.

SUMMARY

The Myers-Bailey modification of the Lewis-Benedict picric acid method of blood-sugar estimation has been further modified to include zinc as a protein precipitant. In the present study the zinc precipitation was found to remove on the average 21 mg. of nonglucose reducing substances. Thus modified the method appears to give results which approach the values for the true sugar content of the blood. The figures reported are still a little higher than those obtained by the Folin-Wu method on the zinc filtrate, but the agreement with the Benedict copper method (II) is close. Certain advantages of the picric acid method of blood-sugar estimation are discussed.

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CONTINUOUS INTRAVENOUS INFUSION*

By Benjamin G. P. Shafiroff, M.D., Brooklyn, N. Y.

IN THE various branches of medicine, particularly in physiology and pharmacotherapeutics, attempts were made to devise an efficient intravenous infusion apparatus. This resulted in the accumulation in our literature of countless numbers of devices, many of which were impracticable and highly complicated. Though many could be applied successfully in animal experimentation, none would satisfy the clinical demands for simplicity and adaptability to human therapy.

As far back as 1913, Dr. Friedemann of the Berliner Institute first reported continuous infusions of various solutions into human beings. He, indeed, is the champion of this method. Following him are a host of clinical observers who reported on the effect of infusions of solutions of adrenalin-saline, Dextrose-Ringer's solution, digitalis, etc. The observations by Dr. Palmedo on the therapeutic results of constant infusions are highly favorable.

We, in this country, have recognized the valuable possibilities of such a method. Our own literature is quite complete on this subject. The name "venoclysis" proposed by Dr. Hendon shows the increasing popularity and consideration it is finding here.

The old method for intravenous infusion was as follows: Under sterile conditions, with a tourniquet around the arm, the median or basilic vein is exposed. The vein is brought up through the wound and separated from the surrounding fat and connective tissue. The distal end of the vein is ligated; into the proximal end is inserted a cannula which is tightly fixed by tying a ligature, which encloses both vein and needle. The cannula is then connected to the apparatus supplying the fluid.

The rate of flow is usually regulated by a pinchcock. The rate of delivery is about 80 to 100 drops per minute. Hendon estimates the average duration of infusion to last from five to eight days without changing the vein. The ideal dose consistent with safety is about 200 c.c. an hour or about 5,000 c.c. per day.

No deaths following this method have been reported. In our own series a patient died two hours after a continuous infusion of 3,000 c.c. of fluid administered during a period of five hours. Autopsy of this case (a carcinoma of the pituitary gland) did not show any morbid effects.

There are two very serious objections to the general mechanical set-up of

*From the Cumberland Hospital, Services of Dr. John E. Jennings, and Service of Dr. Ira I. Kaplan.

the procedure described above. "Cutting down on the vein" is in reality a minor surgical operation, attended with all the usual dangers of infection, shock and trauma. A suitable constant heat apparatus to maintain a temperature of 100-101° is essential. An apparatus eliminating these problems as far as possible will surely increase the demands and the use of this form of therapy.

After a year and a half of trial, we finally adopted the device described below. A Fordyce needle (*G*) is attached to a Luer-Kaufman syringe (*F*).

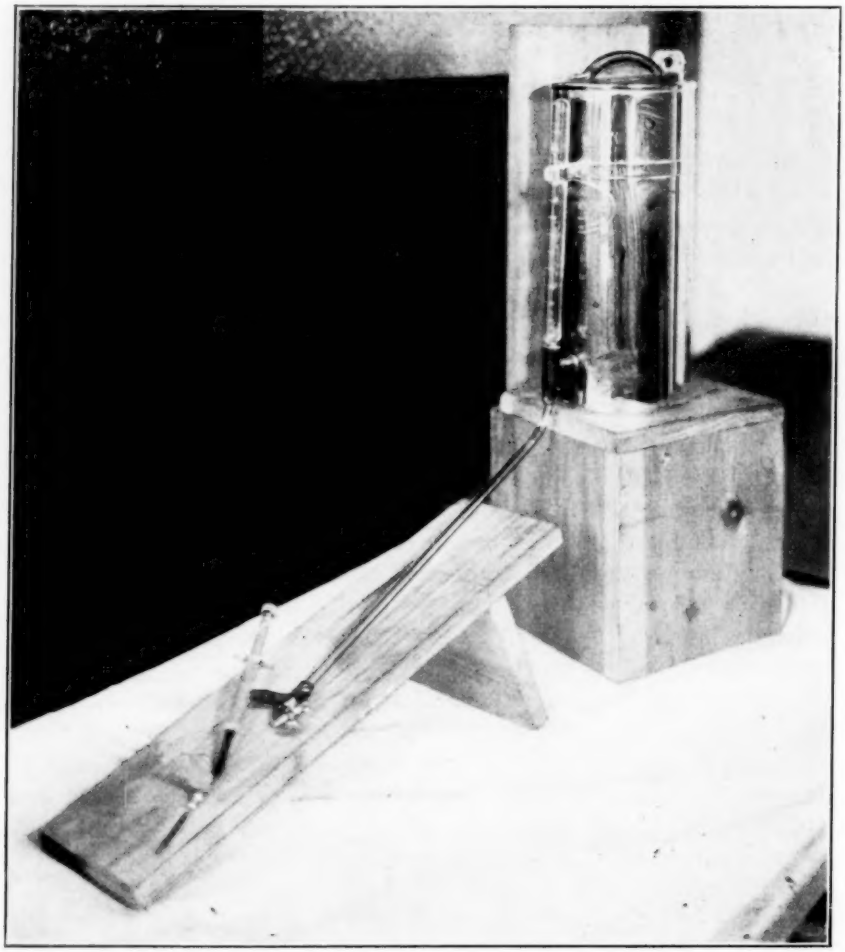

Fig. 1.

(See Figs. 1 & 2.) The syringe is of the usual 2 c.c. type, except for a glass tube inserted into the barrel giving it the apearance of a "T" tube (*F*). The needle is inserted into the vein through the skin; blood is aspirated into the syringe to the level of the side tube. The attachment is then made by a small piece of rubber tubing (*E*). The piston is then withdrawn to a point just beyond the aperture of side tube. As this is done, the driving pressure of the solution forces the fluid into the syringe; the blood is driven back to the vein;

and the slow infusion started. The reservoir (*C*) is a thermos container originally used for the Harris Drip. It can retain its original temperature for several hours. It contains about 1500 c.c. of fluid. A water gauge attached to the aluminum thermos indicates the quantity delivered and serves as a regulator for the rate of flow.

The main stem connecting the tank to the syringe is made of thick-walled glass tube, whose bore is 0.5 cm. Such a tube in itself regulates the velocity of flow. Together with the pinchcock, the rate of flow is under reliable control. The water gauge gives an accurate measure of the amount of fluid in the container as well as the outgoing amount. It disposes of the accessory side burette attachment devised by Freidell. Murphy Drip tubes to show the number of

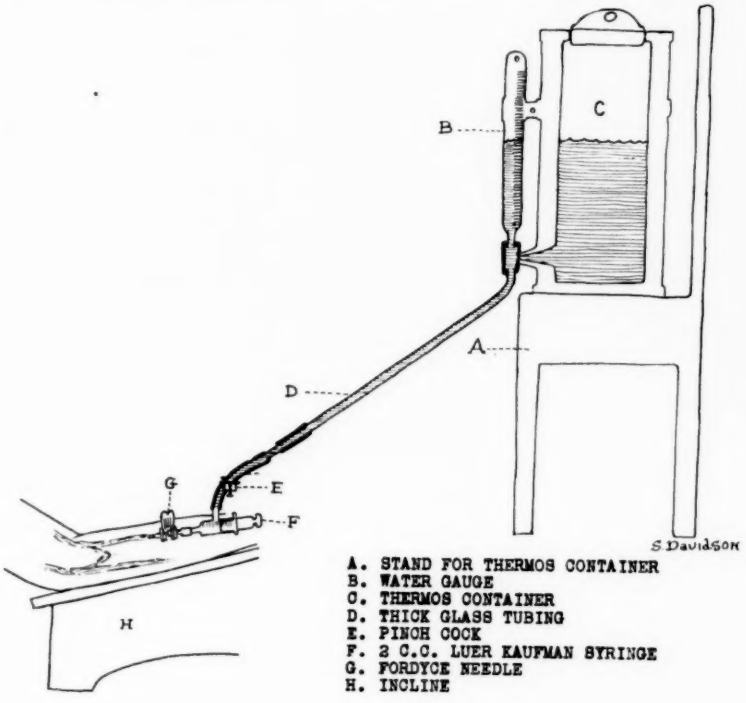

Fig. 2.

drops per minute cannot be used in the constant infusion apparatus. Their construction cuts off the driving pressure from the tank; the pressure in the tube is insufficient to overcome the venous pressure.

The thermos is placed on a simply constructed wooden box, which stands on the patient's table. The box raises the reservoir to a height about 10 cm. This is an interesting innovation. In all the previous methods, the container is hung on a high stand about two feet above the bed.

The arm of the patient is fixed to an incline which pitches toward the heart. This facilitates the flow of the fluid, decreases the venous resistance, and serves as a suitable holder for the arm. The incline is a flat board about 8 inches long, to which at one end is fixed perpendicularly a smaller board 3 inches long (see Fig. 2).

The rubber couplings are sterilized by boiling. The inside of the container is sterilized by flaming with 95 per cent alcohol. The glass tube is boiled. Solutions used for continuous infusion must be completely sterilized. All drugs should be of a high standard purity.

At this point in our report, we propose to review the physiologic principles of intravenous injections. Hooker in his specially constructed apparatus has estimated venous pressure in man to be about 20 mm. of H_2O. To overcome this pressure our container holding 1000 gm. and elevated about 10 c.c. from the vein will exert a pressure of

$$1{,}000 \text{ gm.} \times 10 \text{ c.cm.} = 10{,}000 \text{ gm./square cm.}$$

The facility of flow, though dependent directly on the driving pressure, is also affected by the viscosity of the fluid and the aperture of the tube at the outlet.

That the reservoir is to be elevated as high as possible is so fixed a thought that it is indeed the only constant factor in the numberless variety of features offered with each new constant infusion apparatus.

As this method has improved in simplicity, clinicians discover an increasing number of indications for its use; such conditions are shock, diabetic coma, postoperatively in gastrointestinal surgery, pediatric malnutrition cases, etc. Neoplastic surgery, especially during the administration of radium or x-ray therapy, requires a method such as this. We, at the Brooklyn Cancer Institute, now propose to establish this as routine during the application of radium plaques in the cancerous lesions of the mouth, rectum, or cervix. It has been found a valuable adjunct in treatment of carcinoma of the esophagus and stomach.

CONCLUSIONS

A continuous intravenous infusion apparatus is devised. A method is offered to American physicians for clinical application and further improvement. The apparatus has been made as simple as possible. In select cases excellent results can be obtained.

I wish to thank Miss C. M. Wolf, Supervisor of Operating Division of Cumberland Hospital, for her kind cooperation and assistance. Grateful acknowledgment is made to Drs. Ira Kaplan and John E. Jennings.

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AN APPARATUS FOR CONSTANT INTRAVENOUS INJECTION INTO UNRESTRAINED ANIMALS

By H. R. D. Jacobs, M.D., Chicago, Ill.*

IN ORDER to administer experimental fluids to animals for many days without interruption it is necessary that they be allowed the freedom of their natural movements during the administration. The machines of Woodyatt[1] and Colwell[2] are useful for shorter periods during which the animals may be

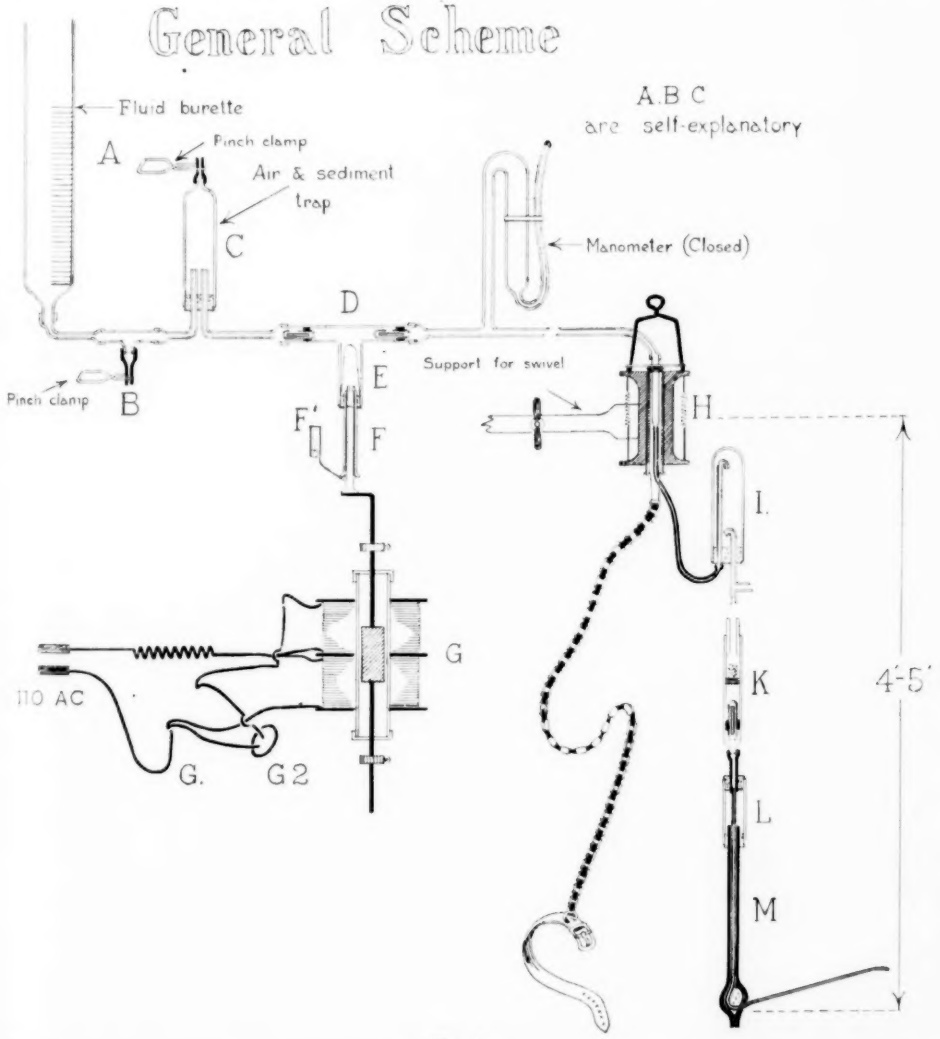

Fig. 1.

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restrained or anesthetized. The apparatus here described was constructed to inject fluids intravenously at a constant rate into dogs which were neither anesthetized nor restrained.

Construction.—The apparatus consists essentially of a pump, a swivel and a cannula, with auxiliary devices. (Fig. 1.)

The pump comprises three parts: (1) valves, (2) diaphragm, and (3) piston-and-cylinder. (Figs. 1 and 2.) The valves, which form the intake and

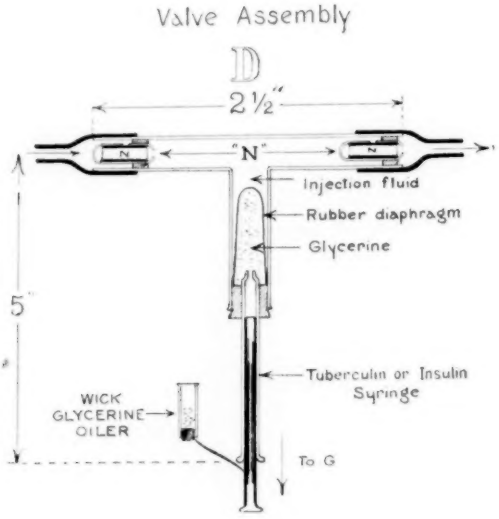

Fig. 2.

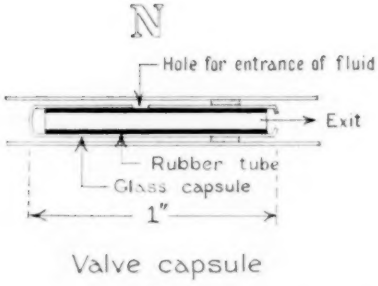

Fig. 3.

exhaust ports of the pump, are two valve capsules (*N*, Fig. 3), one at each port. The diaphragm (*E*), which serves to separate the injection fluid from the fluid of the piston-and-cylinder, is a finger from a rubber glove held between the periphery of a rubber cork and its surrounding glass tube. A good insulin or tuberculin syringe performs well as the piston-and-cylinder. A mixture of glycerine and water lubricates the syringe and occupies the outer side of the diaphragm. A wick "oiler" (*F'*) drops glycerine on the plunger of the syringe.

The motive power for the pump is provided by the magnetic force of two

solenoid coils (each 650 turns No. 24 cotton-covered copper wire) acting alternately upon an iron core. (All but the core should be brass.) The coils are energized by city current, and they are timed by a mechanism whose source of power is a synchronous motor also run by city current. The advantages of this arrangement are that several pumps may be timed by a single central mechanism and that the force activating the pump is flexible and quiet in action (*G*, Fig. 1).

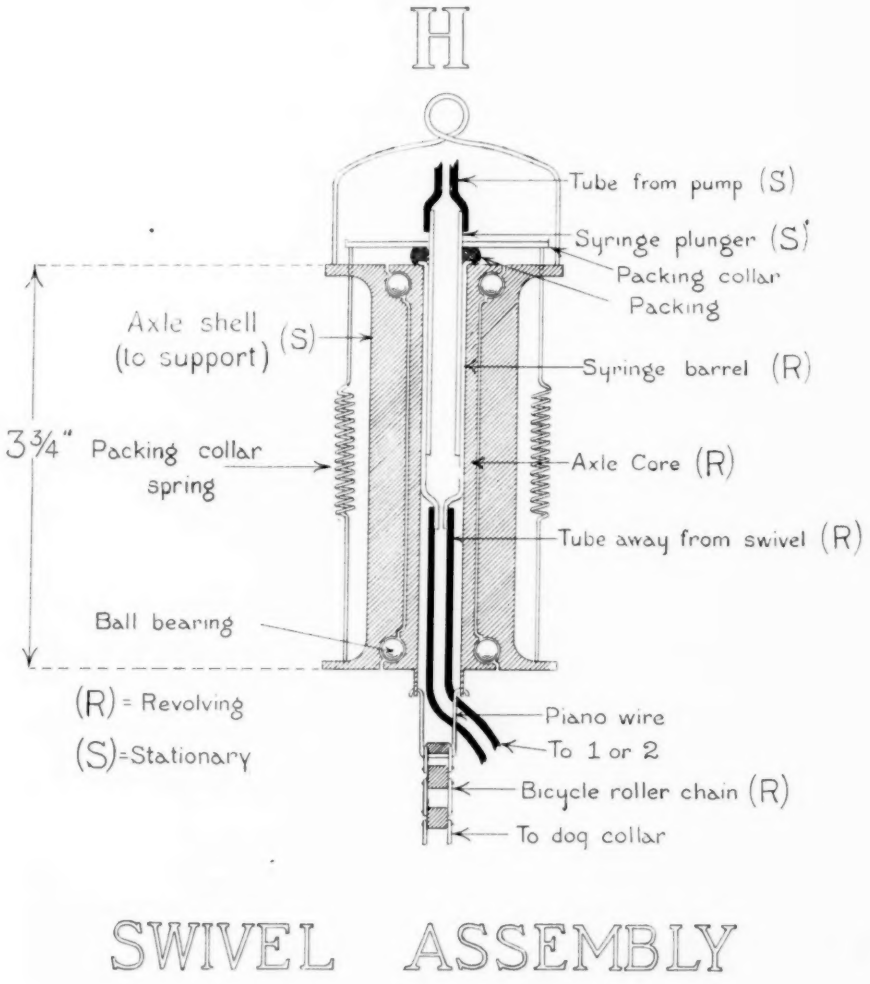

Fig. 4.

A tube from the exhaust port of the pump leads to a closed mercury manometer and thence to the swivel (*H*, Fig. 4).

The swivel, which is the important factor in protracted experiments, allows the animal perfect freedom. It consists of a supporting and a conducting member. The supporting member is the front axle of a motorcycle of the self-contained-bearing type.* The purpose of this axle is to act as a swivel for the

*A satisfactory axle is that made by Eclipse Machine Co., Elmira, N. Y.

chain to which the animal is attached, thereby protecting the conducting system from all stresses. In use it is supported by a strong clamp attached to a substantial fixture about four feet above the animal. The conducting member, which transports the fluid through the swivel, is a small glass syringe, the plunger of which, made into a tube, receives, at its upper end, the tube from the pump and is stationary. The barrel of the syringe is seated snugly inside the core of the axle and rotates with it. (A small ring of vaselined packing material held in place by collar and springs seals the joint between plunger and barrel.)

A tube from the hub of the syringe barrel leads away from the swivel to the air cushion and flow indicator (*I*, Fig. 5), or, in experiments in which great accuracy of measurement or frequent change of the injection fluid is neces-

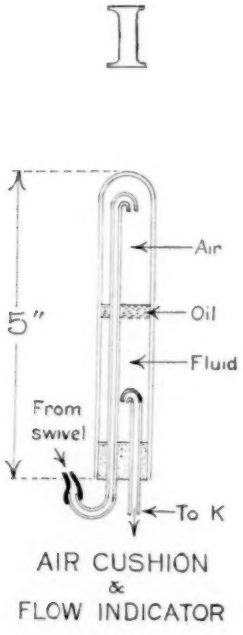

Fig. 5.

sary to the arrangement (*I-2*, Fig. 6). In the latter the injection fluid is separated from the pumping fluid by paraffin oil, and the reservoir is close to the cannula.

From (*I*) or (*I-2*) a tube leads to the check valve or pressure release (*K*, Fig. 7). The pressure release valve contains a valve capsule (*N*, Fig. 3) which offers the desired resistance to the flow of fluid. It is indispensable when the rate of injection is below 10 c.c. per hour but unnecessary when the rate is high. Its function is to establish a positive one-way flow to prevent ebb and flow in the cannula, and, with the aid of the air cushion above, to smooth the fluctuating output of the pump.

A tube from the lower end of the release valve leads to the cannula (*M*, Fig. 8). The cannula is a silk-and-wax ureteral catheter. Its upper end is sealed inside a glass tube to accommodate the tube from the release valve. Its

lower end is free for about five inches, which part lies inside the vein in use. The intervening portion is enclosed by a thick-walled rubber tube which also surrounds the cork disc around which the catheter turns.

The union (*L*, Fig. 8) between the cannula and release valve may be strengthened by a section of larger glass or metal tube supported at each end

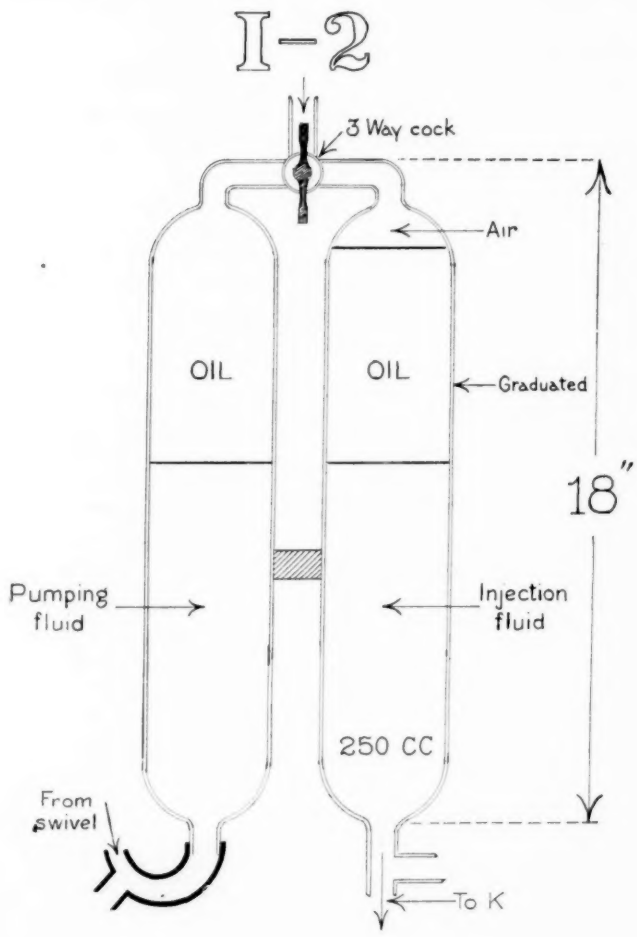

Fig. 6.

by a split cork. Adhesive plaster strips are applied from the cork disc below to beyond the release valve above for stiffness and strength.

A bicycle "roller" chain attached to the lower end of the axle core (with piano wire) runs directly to the dog collar with enough slack to permit the animal to lie down. It supports the air cushion (*I*, and also *I-2* when used) and guides the conducting tube to the animal. A small amount of tension up-

ward upon the lower portion of the chain and injection tube is maintained by means of suitable elastic material to take up slack.

The valve capsule (*N*, Fig. 3) has been found very satisfactory in use. Its construction is evident from the sketch, and it possesses the advantages of long competent life and ease of adaptability to various uses.

It is obvious that tubing should be of small lumen and thick wall, and that all joints between rubber and glass tubes should be wired securely. Air inside the pump will tend to make the rate of injection irregular.

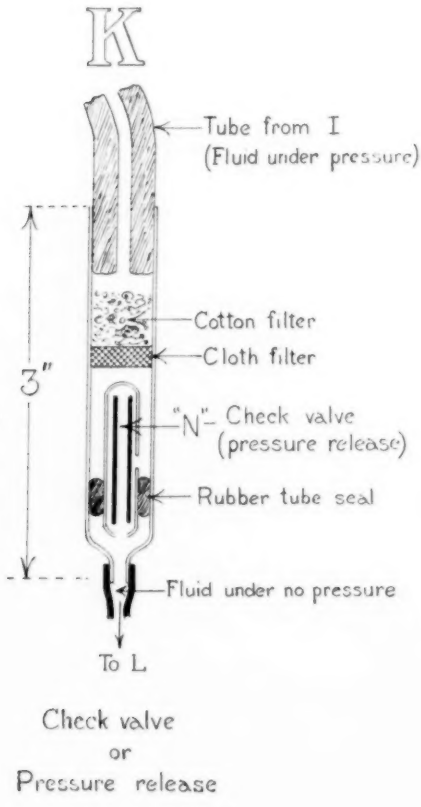

Fig. 7.

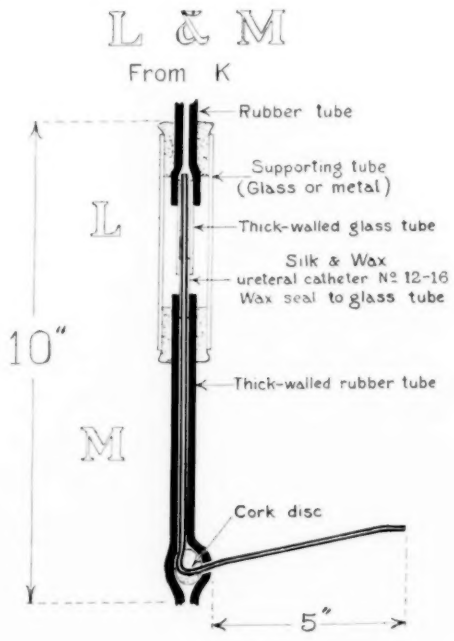

Fig. 8.

Insertion of the Cannula.—Under local anesthesia one jugular vein is exposed near the base of the neck. The vein is ligated above and clamped below, and a longitudinal slit made between the two. One suture is laid above the slit to include the vein and some subcutaneous tissue on each side and one is laid below the slit to include only an outer small segment of the vein's lumen and also subcutaneous tissue on each side. The clamp is taken off and the cannula introduced through the slit toward the heart for four or five inches. The sutures are then tied over the cannula and with one of them an imbrication of more subcutaneous tissue is made over the point of entrance into the vein. The skin is sutured in the usual way. It is best to lay the cannula in the

upper end of the incision. The cork disc and thick-walled rubber tube are then taped securely to the animal's neck and the collar is placed in front of the tape.

Performance.—With proper adjustment this apparatus will inject fluid intravenously at a remarkably constant rate day after day without attention other than casual to keep the reservoir filled. During the injection the animal

Fig. 3.—This photograph shows the degree of freedom which the animal may enjoy during the injection.

has freedom within a circle of a radius of about three feet and may lie down or turn around at will. It is not hampered in any movement. Only the exceptional animal (dog) will disturb the tube enough to require a muzzle.

The rate of injection may be altered by changing the frequency of the action of the piston (ten to forty seconds) or by varying the piston displace-

ment. Five cubic centimeters per hour and upward may be injected satisfactorily. The injection may be continued for weeks. Postmortem examinations of dogs having received experimental fluids by this method for one to three weeks revealed a tube-like thrombus which contained the cannula but did not occlude the vein. The tip of the cannula may lie in the superior vena cava or even in the entrance to the right auricle without causing trouble. It is interesting to note that the cannula rarely becomes occluded.

Mr. Howard Ericson kindly prepared the drawings.

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2. Colwell, A. R.: J. Lab. & Clin. Med. **15**: 556, 1930.

A MODIFICATION OF ROTHERA'S TEST FOR ACETONE BODIES IN THE URINE*

By S. Gordon Ross, M.D., Pasadena, Calif.

ROTHERA'S original test: To 5-10 c.c. of urine in a test tube add a little ammonium sulphate, 2-3 drops of a freshly prepared 5 per cent solution of sodium nitroprusside, and 1-2 c.c. of concentrated ammonium hydroxide. The development of a permanganate color indicates the presence of acetone bodies.

A modification of this method is now suggested whereby one dispenses with the sodium nitroprusside solution. This solution is unstable and must be kept in the dark, preferably in an ice box; otherwise it deteriorates within a week. The suggested modification accomplishes this by combining the sodium nitroprusside and ammonium sulphate in the dry crystalline form, one part of the former, powdered, mixed with 100 parts of the latter.

Routine uranalysis can be speeded up by keeping prepared in the laboratory a large number of test tubes containing approximately one gram of the powdered mixture described above. Five c.c. of urine added to such a tube and agitated before adding 2 c.c. of concentrated ammonium hydroxide accomplish the test.

Large quantities of the powdered mixture may be prepared and kept indefinitely without deterioration. Five years' experience in using this modification has proved its justification. Some technicians prefer a micromethod using 5×50 mm. tubes and one-fifth of each of the ingredients. This latter method may be used in bedside diagnosis because of the small space occupied by the necessary apparatus in the physician's bag.

*Received for publication, October 27, 1930.

THE DETERMINATION OF CHOLESTEROL IN WHOLE BLOOD*

By J. C. Forbes, M.A., Ph.D., and H. Irving, Richmond, Va.

THE authors have recently described a method for the determination of cholesterol in blood plasma and serum[1] which offers several advantages over the older methods. The chief of these is that the whole determination is carried out without the application of heat, thus reducing to a minimum, if not completely eliminating, the possibility of the development of a brown color in the unknown, which so often happens when heat is used and which makes accurate colorimetric readings very difficult. A modification of the method which makes it suitable for the determination of cholesterol in whole blood is here described.

Method.—Place approximately 2 gm. of Doucil, previously dried at 120-125° C., in a 10 c.c. glass stoppered graduated cylinder. The Doucil should reach to approximately the 3 c.c. mark. Add 1 c.c. of chloroform, stopper and shake so as to distribute the chloroform and separate the Doucil particles. Add 0.25 c.c. of distilled water and shake again. Add 0.5 c.c. of blood directly on to the Doucil particles, shake well, and finally add 9 c.c. of chloroform and shake for one to two minutes. Set the cylinder aside, inclining it so as to distribute the Doucil along the tube. Shake occasionally and after approximately three hours filter through a little glass wool. If any of the Doucil pass through the filter, refilter until the chloroform solution is perfectly clear.

Transfer 5 c.c. of the filtrate to another 10 c.c. glass-stoppered cylinder. Measure 5 c.c. of a standard solution in chloroform (containing 0.5 mg. of cholesterol) into a similar cylinder. To each of the solutions add 2 c.c. of acetic anhydride and 0.1 c.c. of concentrated sulphuric acid. Mix by inverting several times, set aside in the dark for fifteen minutes and compare in a colorimeter. If the room temperature is warm, it is advisable to place the cylinders in tap water at 22° C. before setting aside in the dark.

Calculations:

$$\frac{\text{Reading of standard}}{\text{Reading of unknown}} \times 200 = \text{mg. cholesterol per 100 c.c. of blood.}$$

In order to determine the efficiency of the method a number of determinations have been carried out on different bloods using Sackett's method[2] and the one here described. The results of these analyses are given in Table I. Also various amounts of the standard cholesterol solution were added to several tubes after the addition of the blood and the cholesterol content then determined and compared with the theoretical amount present as calculated from the value obtained for the blood itself plus that of the added cholesterol. These results are given in Table II. In Table III a number of determinations

*From the Department of Biochemistry and Medicine, Medical College of Virginia.
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on bloods obtained from normal individuals are recorded. It will be seen that the values fall well within the normal accepted values.

TABLE I

COMPARISON OF CHOLESTEROL DETERMINATIONS BY AUTHORS AND SACKETT'S METHOD

BLOOD NO.	CHOLESTEROL CONTENT, MG. PER 100 C.C.	
	AUTHORS' METHOD	SACKETT'S METHOD
1	206	188
2	268	260
3	189	182
4	178	195
5	202	200
6	113	117
7	160	155
8	159	164
9	125	123

TABLE II

CHOLESTEROL VALUES FOR WHOLE BLOOD AND FOR WHOLE BLOOD PLUS ADDED CHOLESTEROL, EXPRESSED IN MG. PER 100 C.C.

BLOOD NO.	CHOLESTEROL CONTENT			PER CENT ERROR
	ORIGINAL BLOOD	SUPPLEMENTED BLOOD		
		PRESENT	RECOVERED	
1	202	262	276	+5
2	202	302	319	+6
3	232	292	276	−5
4	232	332	324	−2
5	244	344	344	0
6	196	256	242	−5
7	185	285	278	−3
8	208	268	260	−3
9	195	295	295	−0

Average error = −0.8 per cent.

TABLE III

CHOLESTEROL CONTENT OF NORMAL BLOODS BY AUTHORS' METHOD EXPRESSED IN MG. PER 100 C.C.

BLOOD NO.	CHOLESTEROL CONTENT	BLOOD NO.	CHOLESTEROL CONTENT
1	185	13	216
2	208	14	162
3	195	15	178
4	190	16	176
5	146	17	176
6	180	18	164
7	150	19	185
8	156	20	244
9	171	21	224
10	205	22	215
11	139	23	229
12	162	24	198

20 to 24 inclusive were on women, all the rest on men.

Effect of Water on Cholesterol Extraction.—It will be seen from Table IV that a moderate amount of water is necessary for complete extraction of the cholesterol. It is, however, not necessary to add any water when the cholesterol content of blood is being determined as the blood itself seems to have enough water for this purpose. Water may, however, be added to

the extent of 0.4 c.c. without interfering with the extraction. Although the addition of water is not necessary in this case, the authors recommend using 0.25 c.c. since its presence aids in the separation of the Doucil particles after the addition of the blood thus favoring extraction.

TABLE IV

EFFECT OF WATER ON CHOLESTEROL EXTRACTION

Part (a) Extraction From Standard Solutions

Tubes 1a and 1b contain Doucil + 5 c.c. $CHCl_3$ + 5 c.c. of standard solution
Tubes 2a and 2b contain Doucil + 5 c.c. standard + 5 c.c. $CHCl_3$
Tubes 3a and 3b contain Doucil + 0.5 c.c. H_2O + 5 c.c. standard + 5 c.c. $CHCl_3$
Tubes 4a and 4b contain Doucil + 0.5 c.c. H_2O + 5 c.c. $CHCl_3$ + 5 c.c. standard
Tubes 5a and 5b contain Doucil + 1 c.c. $CHCl_3$ + 0.5 c.c. H_2O + 5 c.c. standard + 4 c.c. $CHCl_3$
Tubes 6a and 6b contain Doucil + 1 c.c. $CHCl_3$ + 5 c.c. standard + 4 c.c. $CHCl_3$

	Number of Tubes											
	1A	1B	2A	2B	3A	3B	4A	4B	5A	5B	6A	6B
Recovered cholesterol, per 100 c.c., mg.	88	89	96	90	100	100	100	99	100	100	88	87
Theoretical	100	100	100	100	100	100	100	100	100	100	100	100
Per cent recovered	88	89	96	90	100	100	100	99	100	100	88	87

Part (b) Extraction From Whole Blood

DETERMINATION	WATER ADDED C.C.	RECOVERED CHOLESTEROL PER 100 C.C. MG.
1	0.00	200
	0.15	204
	0.25	205
	0.25	208
	0.40	206
	0.60	136
	0.70	104
2	0.00	166
	0.10	160
	0.10	160
	0.25	160
	0.40	156
	0.40	158
3	0.00	344
	0.25	344
4	0.00	156
	0.25	152

Time Required for Extraction.—The time required for the extraction of the cholesterol is undoubtedly influenced by the degree and the duration of shaking. It will be seen from Table V that under the conditions described complete extraction is obtained in less than three hours.

TABLE V

TIME OF EXTRACTION	CALCULATED MG. OF CHOLESTEROL PER 100 C.C. OF BLOOD
18 hr.	138
18 hr.	139
180 min.	141
140 min.	137
140 min.	138
110 min.	124
90 min.	118

DISCUSSION

In carrying out determinations of cholesterol by this method the direction given should be carefully followed. It is very important that 1 c.c. of chloroform be added before the blood for otherwise complete extraction cannot be obtained in the time recommended. This is likely due to the blood entering deeply into the Doucil particles, unless the pores have been previously more or less filled with chloroform, thus making extraction very slow. The need of a certain amount of water may be explained on the probable assumption that the Doucil has a greater attraction for water than for chloroform and consequently the water would tend to displace the chloroform from the pores of the Doucil thus preventing absorption of the cholesterol which is chloroform soluble but water insoluble.

SUMMARY

Forbes and Irving's method for the determination of cholesterol in blood plasma and serum has been modified so that it is applicable for whole blood analyses. The method has been compared with Sackett's method and found to give agreeing results.

The Doucil used in these experiments was kindly supplied by the American Doucil Co., Philadelphia. It is also a pleasure to acknowledge a research grant from the Chemical Foundation which enabled the senior author (J. C. F.) to devote most of his time to research.

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1. Forbes, J. C., and Irving, H.: Determination of Cholesterol in Blood Plasma and Serum, J. Lab. & Clin. Med. **16**: 520, 1931.
2. Sackett, G. E.: Modification of Bloor's Method for Determination of Cholesterol in Whole Blood or Blood Serum, J. Biol. Chem. **64**: 203, 1925.

A NEW TIME MARKER*

By Felix P. Chillingworth, M.D., Boston, Mass.

IN PHYSIOLOGIC and pharmacologic studies the time factor is important. The types of apparatus used for timing vary from those necessitating mercury contacts (pendulum clock-metronome) to the more complicated master clocks.

These methods are excellent but expensive and require much attention and are by no means foolproof in the hands of students.

To simplify these existing conditions I have utilized the small electric clock which with its synchronous motor operates on an alternating current of definite frequency. This frequency is usually sixty cycles, hence the speed of the motor is constant and uniform.

*From the Department of Pharmacology, Tufts College Medical School.
Received for publication, December 5, 1930.

By the addition of two binding posts and spring contacts the shaft of the electric clock will, when electrified by a dry cell, yield current every second. This current will operate a signal magnet until either the dry cell or the public utility runs down. Furthermore this second current can be used for the operation of an inductorium. With the use of relay keys accurate time can be supplied to many laboratory tables.

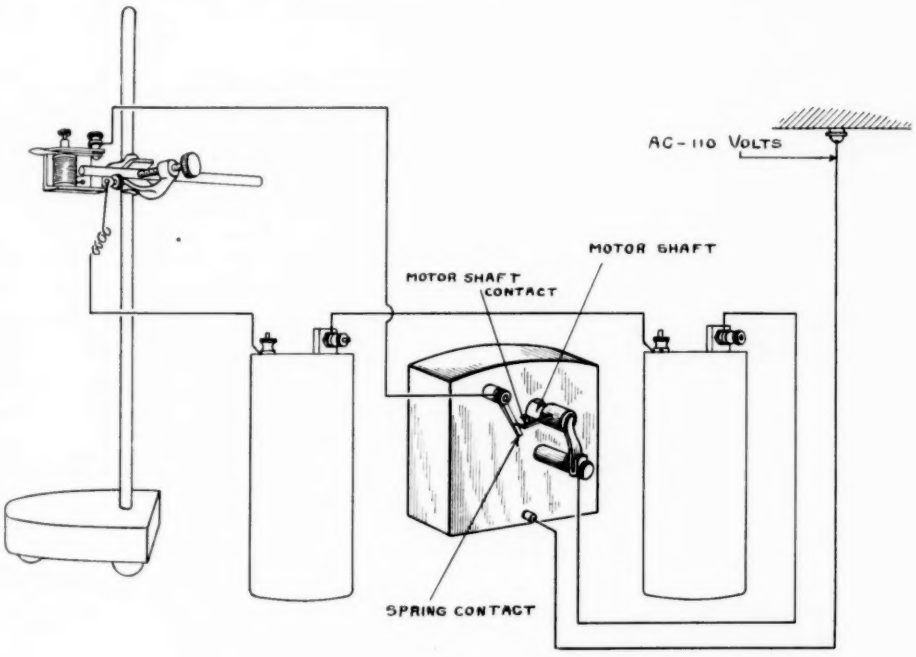

Fig. 1.

Any desired time unit can be obtained by using simple gearing.

Fig. 1 gives in detail the necessary alterations.

A time marker as described above has been in use in this laboratory for over a year and has proved in all ways satisfactory. The cost depends upon the type of electric clock selected.

TWO IMPROVED DESIGNS OF THE PEEBLES-LEWIS CLINICAL COLORIMETER*

By Robert C. Lewis, Ph.D., Denver, Colo.

IN 1918 Peebles and Lewis[1] described a simple colorimeter which had proved surprisingly accurate in their hands and when used by students. Since that time we have used this instrument or an improved model in our classes in biochemistry with great satisfaction, the comparatively low cost making it possible for each student to have one exclusively for his own use. During the past five years the student model of this colorimeter has been in use in our laboratory for all of the colorimetric work in blood chemistry done by us for the Hospitals and Out-Patient Clinic of the University of Colorado School of Medicine. Standard methods for the colorimetric determinations of the chemical constituents of the blood have been modified[2] for use with this colorimeter in such a way that dilutions and color development are made in the tubes of the colorimeter. Consequently, a great saving in the amount of apparatus used has been accomplished and the routine work of the blood chemistry laboratory has been thereby greatly facilitated. All of this has been accomplished without the sacrifice of accuracy.

The original colorimeter was made from a Pillsbury slide box designed to hold twenty-five microscopic slides, two matched test tubes graduated to 30 c.c. in 0.2 c.c. divisions, and a ground glass slide. One end of the box was entirely removed and in the other end two holes were bored large enough to admit two test tubes. The box was painted black inside and outside, the ground glass slide was placed in one pair of grooves near the open end of the box, the tubes were placed in the holes so that they rested on the ground glass slide, and the instrument was ready for use, daylight being reflected up through the tubes by a mirror placed on a laboratory table at an angle of about 45 degrees.

The purpose of the present paper is to describe two improved models of this simple colorimeter. The first, the student model, was designed about eight years ago and has been used continuously in the student laboratory and for routine blood chemistry during that time. Its accuracy has been demonstrated repeatedly to be equal to that described by Peebles and Lewis[1] for the original slide box model. The second improved colorimeter, the laboratory model,† has been recently designed particularly for use in hospital and clinical laboratories where constant use makes greater durability desirable.

*From the Department of Biochemistry, University of Colorado School of Medicine.
Received for publication, December 27, 1930.

†Both this and the student model may be purchased from the Denver Fire Clay Company, 1746 Champa Street, Denver, Colorado.

The student model (Fig. 1) differs from the original slide box colorimeter only in a few respects. The principal change is that the box has been made larger, its dimensions being 21 cm. × 8.2 cm. × 4.2 cm. as compared to 17 cm. × 8.3 cm. × 3.7 cm. for the slide box. With this greater length, the mirror or, to be more accurate, the reflector can be incorporated in the instrument. A thin piece of wood, 6.2 cm. × 10.2 cm., is fastened to one end of the box to provide an enlarged base on which the colorimeter box will stand securely. As in the original model, the top has two round holes 18.5 mm. in diameter, one on each

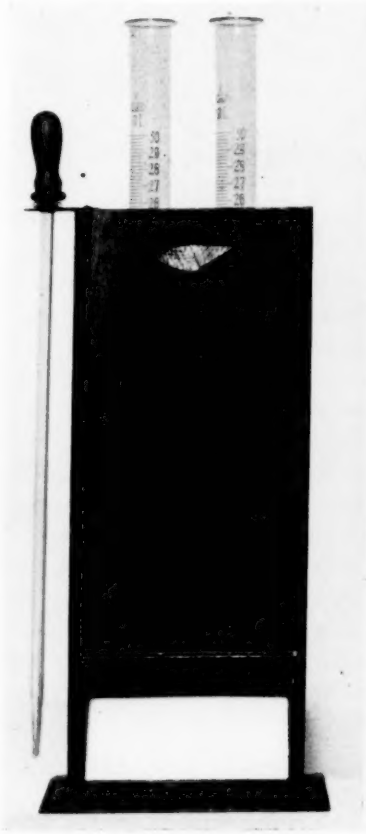

Fig. 1.—Student model.

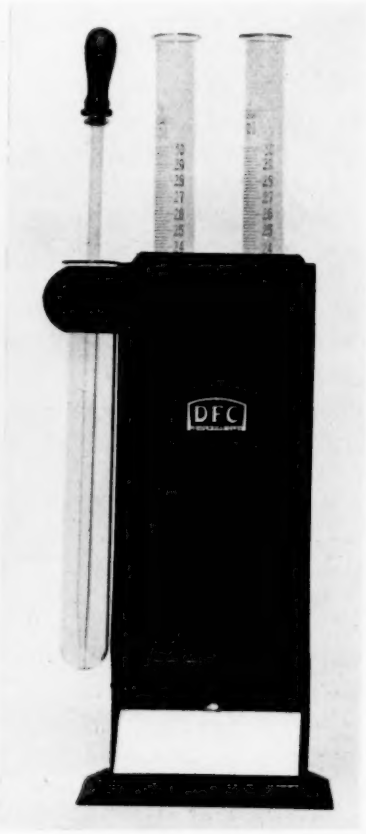

Fig. 2.—Laboratory model.

side of the center, to receive the two graduated tubes. The adjacent edges of these holes are 15 mm. apart. A ground glass slide, 76 mm. × 38 mm., fits in grooves parallel to the ends on either side of the box and 4.5 cm. from the bottom. Hence, the glass slide may be easily replaced, should it be broken. The removable front of the box is held in place by grooves in the side of the box and may be removed by sliding it out at the top of the colorimeter. It is shorter than the box itself and, when in place, rests on the ground glass slide, leaving an opening in the bottom part of the front of the colorimeter for the reflector. This reflector consists of a piece of an ordinary index card tipped at an angle of 45 degrees, so that it extends from the front of the bottom to the

back of the colorimeter box a little below the bottom of the glass slide. The presence of the reflector as a part of the colorimeter adds greatly to one's convenience in using the colorimeter. Two matched 30 c.c. test tubes graduated in 0.2 c.c. divisions, similar to those used in the original model, and a dropping pipette with glass part 21 cm. long complete the colorimeter. The graduated test tubes used are 23 cm. long and 14.5 mm. inside diameter.

The laboratory model (Fig. 2) is similar to the student model, although somewhat smaller, and is made of metal with black crystalline finish. The reflector is made of white opal glass, so that it may be readily cleaned with a damp cloth. An added improvement is a clip on the right side of the instrument to hold a plain test tube for receiving the fluid removed from the "standard" tube of the colorimeter when the colors in the two tubes are being matched.

Although designed for use in the doctor's or hospital laboratory, this more substantial model of the colorimeter might well be used as student equipment. Its relatively small price, as compared to that of the more expensive colorimeters commonly used, makes possible individual student equipment at a cost that is not prohibitive. The advantage of having an accurate colorimeter for each student is too obvious to require further emphasis.

The technic for use of the colorimeter for blood chemistry determinations is exactly the same for any one of the models described. Blood filtrate (Folin and Wu[3]) and standard solution are placed in the "unknown" tube and in the "standard" tube, respectively, in such quantities that there will be approximately one-half as much of the material to be determined in the former as in the latter. One-half as much reagent is added to the "unknown" tube as to the "standard" tube and the contents of the two tubes are diluted to 10 c.c. and 20 c.c., respectively. The contents of the "unknown" tube are mixed by agitation without inverting the tube; and those of the "standard" tube, by inversion after the thumb has been placed over the mouth of the tube. By this procedure, the concentration of reagents in the two tubes will be the same and the color developed in the two tubes will be somewhat near the same intensity per unit volume. The "unknown" tube is always placed at the left; and the "standard" tube, at the right in the colorimeter. Solution is removed from the "standard" tube into a plain dry test tube until its color matches that of the "unknown" tube. Matching is accomplished by holding the instrument about two feet from the eye and looking down through the tubes with one eye, first at one tube and then, by slightly tipping the colorimeter, at the other tube. A check reading is made by starting with too little solution in the "standard" tube and adding solution until a match is obtained. Further readings are made alternately as described above.

The average of three or four successively consistent readings is taken as the figure on which to base the calculation of results, which may be easily made by use of the following formula: $\frac{B \times \text{average reading} \times 100}{C} = \text{mg. of}$ A per 100 c.c. of blood, where A is the constituent which is being determined, B represents the mg. of A per c.c. of colored solution in the "standard" tube, and C equals the c.c. of blood represented by the filtrate in the "un-

known'' tube. For example, if blood sugar is being determined by the method of Folin and Wu[4] as modified by us[2] for the colorimeter herein described, 1 c.c. of standard glucose solution, equivalent to 0.08 mg. of glucose, and 0.4 c.c. of Folin-Wu filtrate would be used in the ''standard'' tube and in the ''unknown'' tube, respectively. Thus, the values of the formula become:

$$B = \frac{0.08}{20} = 0.004, \text{ and } C = \frac{0.4}{10} = 0.04.$$

If the average reading of the ''standard'' tube is 9.6, we have by substitution in the above formula:

$$\text{Mg. of glucose per 100 c.c. of blood} = \frac{0.004 \times 9.6 \times 100}{0.04} = 96.$$

As mentioned in the beginning of this paper, colorimetric determinations of the constituents of blood may be made with this instrument in such a way that dilutions and color development are made in the tubes of the colorimeter, thereby accomplishing a considerable saving in apparatus used as well as in time required for each estimation. Extra pairs of graduated tubes may be purchased for use with the colorimeter. By having a number of pairs of matched tubes, one for each determination to be made in any one day, several determinations may be set up at one time and considerable saving in the time required for routine hospital blood chemistry will result.

Other colorimetric determinations than those of blood chemistry may be performed readily with this colorimeter. Phenolsulphonephthalein determinations for kidney function and the estimation of the various metabolic constituents of urine are some of the uses to which the instrument may be put.

SUMMARY

1. A simple, accurate colorimeter is described by the use of which the standard colorimetric methods of blood chemistry are considerably simplified and routine hospital laboratory work in blood chemistry is thereby greatly facilitated.

2. By employing this colorimeter as individual equipment, colorimetric methods for the determination of the metabolic constituents of urine as well as of blood may be taught to a class of medical students to much better advantage than where a few expensive colorimeters constitute the entire facilities for color comparison.

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A QUANTITATIVE TEST FOR THE DETERMINATION OF XANTHINE AND HYPOXANTHINE IN THE BLOOD. PRELIMINARY REPORT*

By Warren H. Cole, M.D., William H. Ellett, M.D., and Nathan A. Womack, M.D., St. Louis, Mo.

A GREAT amount of work has been done on the uric acid content of the blood and urine, but very little work has been reported regarding the immediate precursors of uric acid, xanthine and hypoxanthine, which are presumably converted into uric acid by the action of an enzyme. This enzyme is xanthine-oxidase, which, according to Miller and Jones[1] and Wells and Corper[2] is found in the human body only in the liver. Morgan[3] has conducted a series of experiments on a number of animals determining the absence or presence of xanthine-oxidase in various organs, and has reviewed the findings of other workers. Apparently nobody has been able to demonstrate the presence of the enzyme in any organ of the human body except the liver, and possibly the small intestine. On account of the ingestion of xanthine-oxidase in our food it is not surprising that a trace is found in the small intestine.

Of the very few methods heretofore described for estimation of purine bases, the one devised by Graves and Kober probably is the most practical except for the determination of uric acid. Graves and Kober[4] have extended the application of their test to the determination of the purine bases in the blood. However, their work on the amount of xanthine and hypo-xanthine in the blood is of a qualitative nature and confined to sheep, which they remark is a very suitable animal to use in the development of the method because of the low content of purine bases contained in the blood. The method described by us in this report has the advantage of simplicity except that a period of incubation for twenty-four hours is required. In this work we have been concerned only with the determination of xanthine and hypoxanthine in the blood.

Working on the assumption that the presence of xanthine-oxidase in the liver of the human body signified that it was produced in that organ, we felt that quantitative determinations of the enzyme in the blood might furnish an index of hepatic function. We, therefore, attempted to derive a test for the enzyme in the blood. Two procedures were available for the detection of the enzyme: (1) the methylene blue technic, first used in the study of oxidizing enzymes by Thunberg[5] in 1920. When xanthine or hypoxanthine is mixed with xanthine-oxidase in the presence of methylene blue, the methylene blue is reduced to its leuco form as the xanthine bases† are oxidized to

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†We wish to call attention to the fact that when the term "xanthine bases" is used in this report, we refer only to xanthine and hypoxanthine.

uric acid. This furnishes a definite color reaction for the enzyme if the xanthine bases are known to be present in pure form. The method was extensively used by Dixon and Thurlow in ascertaining the distribution of xanthine-oxidase in the tissues of the human body. (2) The detection of the amount and rapidity of production of the uric acid formed when blood is incubated in the presence of a known amount of xanthine or hypoxanthine. By the use of both these procedures, however, we succeeded only in confirming the findings of Brugsch and Schittenhelm,[6] who were unable to detect any xanthine-oxidase in the blood of human beings. We, therefore, abandoned the hope of deriving a test for the enzyme in the blood and transferred our attention to the problem of devising a method for the determinations of xanthine and hypoxanthine in the blood by converting the xanthine bases to uric acid by adding an excess of xanthine-oxidase. We are of the opinion that the increase in amount of uric acid found in the blood sample after addition of xanthine-oxidase and sufficient incubation, over the amount found after withdrawal from the vein, is best explained by conversion of xanthine and hypoxanthine to uric acid.

Morgan, Stewart and Hopkins[7] discovered that the enzyme was present in large amounts in cow's milk. A year or so later, Dixon and Thurlow[8] prepared an extract from milk which contained the enzyme in a high concentration of quite stable character, and which was extensively used by them in the determination of the dynamics of enzyme action. This active material was prepared by Dixon and Thurlow as follows: "The activity of the milk is first tested in the usual way with a small trace of xanthine and methylene blue. The milk is then mixed with an equal volume of saturated ammonium sulphate solution and allowed to stand a few minutes. The precipitated caseinogen and fat are then filtered off and dried as far as possible by pressing between filter papers. The ease of the subsequent manipulation depends to a great extent upon the thoroughness with which this is carried out. The mass is now broken up and extracted thoroughly with cold ether, the process being hastened by frequent shaking. The ether is drained off and the material again pressed between filter papers so as to remove as much ether and water as possible, after which it is spread out and dried in a vacuum desiccator for about twelve hours. After this it is again extracted with ether, dried thoroughly in vacuo, and powdered in a mortar." The bulk of this material consists of caseinogen along with the xanthine-oxidase. It is freely soluble in water, and will retain its activity for six months if kept in a brown glass bottle at ice box temperature.

The incubation of a sample of blood containing a small amount of this powdered material produces a marked increase in the amount of uric acid recoverable from the sample. This fact was made use of in the employment of the following routine procedure: To each of two test tubes 2 c.c. of oxalated blood are added and in *one* of these is placed 50 mg. of the active material containing xanthine-oxidase. Both tubes are then incubated at 37° C. for twenty-four hours. After incubation, the amount of uric acid in each sample is determined by the Benedict[9] colorimetric method. The xanthine-

oxidase which is added, converts the xanthine and hypoxanthine of the blood to uric acid. Theoretically, it should make no difference whether the determination of uric acid in the control sample was made before or after incubation since we found that incubation of oxalated blood alone did not alter the uric acid content. It seemed more convenient, however, to incubate the control sample along with the sample to which enzyme had been added, and make the determinations at the same time, after incubation.

By this procedure it was found that the ratio of xanthine base to uric acid in the blood of normal individuals is approximately two to one, and that there is a variation of this ratio in various pathologic conditions, though our series is not yet sufficiently extensive to evaluate these variations with certainty.

The blood for these experiments was collected under sterile precautions, but no attempt was made to maintain sterility during the incubation. After twenty-four hours, which was found to be the maximum time interval necessary for conversion of xanthine base to uric acid, no gross evidence of putrefactive changes were observed. After forty hours, however, there was evidence of putrefaction, including foul odor, etc.

It will be noted in Table I, that our readings on supposedly healthy individuals, who presented no clinical reasons for having an increase in the amount of uric acid in their blood, are well above the variations of 1.5 to 3.0 mg. per 100 c.c. of blood, which are given by many authorities. We, therefore, agree with Lennox and O'Connor[10] who have made extensive investigations regarding the normal amount of uric acid in the blood, and recommended that the upper limit of normal be shifted from 3.0 mg. per 100 c.c. to a level as high as 5.0 mg. In a series of 20 known normal individuals, their figures varied from 3.3 to 5.7 mg. with an average of 4.5 mg. per 100 c.c. of blood. We were unable to determine whether they used the Folin or Benedict method in this series, but it makes very little difference, since their analyses revealed results by the Folin method to be only 0.2 mg. higher than by the Benedict. They also noted that fasting patients had an average reading 0.5 mg. higher than the nonfasting group.

CONTROL TESTS CONDUCTED ON THE PROCEDURE

1. Incubation of the enzyme alone produced no uric acid and incubation of oxalated blood in the presence of boiled enzyme solution produced no increase in the uric acid content of the blood sample.

2. Variations in the amount of enzyme material (beyond a minimal quantity of 10 mg.) added to a blood sample, produced no effect on the amount of uric acid converted from xanthine and hypoxanthine. Weighed quantities (from 10 to 100 mg.) of the active material were found to produce the same amount of additional uric acid when added to 2 c.c. of blood.

3. Variations in time of incubation were found to be important. An incubation period of less than twenty-four hours was found to be insufficient to oxidize all of the xanthine and hypoxanthine in the blood to uric acid. Further incubation up to forty-eight hours produced no change.

4. Known amounts of xanthine were converted to uric acid with 96 per cent yield when incubated with enzyme for twenty-four hours at 37° C. This temperature was used throughout the series of experiments. Variation of incubation temperature between 27° C. and 37° C. produced no change in the amount of converted uric acid, if the incubation period was twenty-four hours or greater. Incubation at ice box temperature strongly inhibited the conversion of the xanthine bases to uric acid.

5. Aeration of either sample of blood during incubation had no effect on the amount of uric acid found. Whole oxalated blood, fully oxygenated was used in all determinations. Incubation of the control blood sample to which no enzyme had been added, also produced no effect on the amount of uric acid.

6. On repeated occasions we found that excess quantities of enzyme when added to a tube containing water and a known amount of xanthine,* would convert all the xanthine to uric acid. The xanthine solutions were made very slightly alkaline because Dixon and Thurlow claim they are unstable when kept in neutral solution. They state that "if neutralized and allowed to stand for several days, a change is found to take place, especially if phosphate is present. They become altogether incapable of oxidation by the enzyme." Five separate experiments were carried out, using altogether 0.8 mg. xanthine. After incubation at 37° C. for twenty-four hours in the presence of the enzyme we obtained 0.854 mg. uric acid as found by the Benedict method. This represents a yield of 96.2 per cent. Since oxygen is consumed during the conversion of xanthine (molecular weight 152) to uric acid (molecular weight 168), 0.888 mg. uric acid would constitute 100 per cent yield. Since we used the same technic of conversion of xanthine bases to uric acid in the blood, we feel it is safe to assume by analogy, that the yield in the blood should also approximate 90 to 100 per cent.

SUMMARY

We have made quantitative determinations of xanthine and hypoxanthine in the blood, utilizing the Benedict method for determination of uric acid before and after the xanthine bases are converted to uric acid by the action of xanthine-oxidase. Most of the details of the procedure we have described have been worked out previously by various workers, but so far as we know, this represents a new application of those principles. Previously, the determination of xanthine and hypoxanthine has perhaps been too complicated to be adopted for clinical use. The variability of the ratio between the amounts of uric acid and xanthine base in the blood of various patients suggests that the amount of xanthine and hypoxanthine has a different significance from that of the uric acid. Our series has been too limited to allow us to form any definite conclusions regarding the true clinical significance, if such is present. On the one occasion when we had an opportunity to follow a patient with exophthalmic goiter under iodine therapy, the

*The xanthine used in these experiments was obtained from the Eastman Kodak Company, Rochester, N. Y. It was used from the original container without chemical treatment of any kind except the addition of a very small amount of alkali, as described in the text.

ratio of the uric acid converted from xanthine base to the uric acid already present, decreased markedly (see Table I) as the preoperative iodine therapy progressed.

TABLE I

TABULATION OF RESULTS IN VARIOUS DISEASES*

DIAGNOSIS	A URIC ACID. MG. PER 100 C.C. BLOOD	B TOTAL URIC ACID. MG. PER 100 C.C. BLOOD AFTER INCUBATION WITH ENZYME	C INCUBATION IN AMOUNT OF URIC ACID. MG. PER 100 C.C. BLOOD	RATIO OF C TO A
Paresis	3.8	13.3	9.5	2.4 to 1
Sarcoma, leg	2.4	10.0	7.6	3.1 to 1
Fracture of os calcis	4.0	17.7	13.7	3.4 to 1
Carcinoma of breast	4.0	11.3	7.3	1.8 to 1
Granuloma inguinale	5.3	14.5	9.2	1.7 to 1
Carcinoma liver (?)	3.8	12.7	8.9	2.3 to 1
Tuberculosis of knee	4.0	14.3	10.3	2.6 to 1
Foreign body, leg	5.0	15.0	10.0	2.0 to 1
Osteoma (?)	4.8	15.0	10.2	2.1 to 1
Encephalitis	4.5	14.5	10.0	2.2 to 1
Infection of the face	5.5	15.0	9.5	1.7 to 1
Carcinoma of jaw, infection	5.0	15.0	10.0	2.0 to 1
Hypertension	4.6	13.3	8.7	1.9 to 1
Arthritis, hypertension	10.6	17.7	7.1	0.6 to 1
Jaundice (obstructive?)	3.0	15.0	12.0	4.0 to 1
Jaundice (obstructive?)	5.7	14.5	8.8	1.5 to 1
Jaundice (obstructive?)	6.4	17.0	10.6	1.6 to 1
Polycythemia vera (?)	4.2	13.3	9.1	2.2 to 1
Chronic nephritis	10.0	20.0	10.0	1.0 to 1
Myoma uterus, before breakfast	4.8	12.0	7.2	1.5 to 1
Same case, 3 hours after lunch	3.3	13.3	10.0	3.0 to 1
Exophthalmic goiter. B. M. 22. Lugol's for 8 days	6.6	20.0	13.4	2.0 to 1
Exophthalmic goiter. Lugol's for 5 days	3.3	13.3	10.0	3.0 to 1
Exophthalmic goiter. Lugol's for 10 days	4.4	16.0	11.6	2.6 to 1
Exophthalmic goiter. B. M. 75. Before Lugol's	3.4	16.0	12.6	3.7 to 1
B. M. 72. Lugol's for 5 days	5.0	15.0	10.0	2.0 to 1
B. M. 45. Lugol's for 12 days	5.0	11.8	6.8	1.3 to 1

*Duplicate analyses conducted on various specimens of blood yielded results which checked within 10 per cent of each other.

It should be stated that we have no definite proof that the uric acid produced by incubation in the presence of xanthine-oxidase arises from hypoxanthine and xanthine. We do feel, however, that the explanation stated above seems to be the most logical one since uric acid can be produced from xanthine in at least 95 per cent yield by incubation in the presence of the enzyme material, and because of the other control tests mentioned. Our conclusions are also dependent upon the supposition that the blue color as produced in the Benedict test for uric acid is actually due to uric acid. Although there perhaps may not be obvious proof that this supposition is true, we are inclined to interpret the blue color reaction, as most authorities do, as being produced by uric acid.

We wish to take this opportunity to express our appreciation to Drs. P. A. Shaffer, Ethel Ronzoni and E. S. West, who have kindly offered their advice in this work.

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THE ROSE BENGAL TEST FOR LIVER FUNCTION*

An Historical Sketch and an Improved Technic

By G. D. Delprat, M.D., F.A.C.S., and W. P. Stowe, M.D.,
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IN THE summer of 1918 an opportunity was offered one of us (Delprat) by Professor Herbert M. Evans, Department of Anatomy, University of California, to study the fate in the body of some three hundred dyes of the triphenylemethane series from his large collection. Rats were used in these experiments and a number of dyes found which, after intraperitoneal injection, were eliminated in the stools, but not in the urine. These dyes were further studied (by intravenous injection) in bile-fistulae dogs, that were given by Dr. George H. Whipple, then Director of the Hooper Foundation of Medical Research. It was noted that many dyes, which in the rat are eliminated only in the stools, appeared in the dog in both the bile and in the urine. The action of rose bengal, however, was constant. It appeared, both in the rat and in the dog—and later in human subjects, constantly and only in the bile. Efforts were made to estimate the quantity of the dye that could be recovered in the bile after intravenous injection. No method was found of separating the dye from the bile pigments, however, nor were studies on normal dogs (where the dye could be recovered from the duodenum with a duodenal tube) satisfactory.

In 1922, while a student in the Medical Department of the University of California, an opportunity was given by Dr. William J. Kerr to continue this

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work. At this time the rate of disappearance of the dye from the blood stream was investigated; and the effect noted on the disappearance time of experimentally produced liver injury. The results of this study have been published.[1] Just before this work went to press, interestingly enough, the independent work of Rosenthal[2] appeared in which the disappearance time was noted of phenoltetrachlorphthalein after intravenous injection. It was with some hesitation in 1923, that we injected this dye into our first human subject. The patient, a Chinaman with a carcinoma of the pancreas with complete biliary obstruction, showed no toxic effects after the intravenous injection of 100 mg. of the dye. In fact, while observing with interest the injection of this beautifully colored "medicine" he stated that he felt much better and wanted more. Frequent injections on this patient gave no toxic effects and led to the subsequent injection of some two to three hundred others.[3]

In our earlier work elaborate calculations were thought necessary to arrive at the figure which would indicate the functioning power of the liver. The fallacy of this was first suggested by Max Dunievitz and N. N. Epstein.[4] Since that time it has been our custom to compare the "two-minute sample" with the "eight-minute sample" as explained below. A slight hemolysis in one or both of the samples with resulting imperfect color match has been a constant source of annoyance. Through the acetone precipitation method (Bloom and Rosenan[5]) suggested by Stowe, this difficulty appears to have been surmounted and the present technic of the test as we are using it now follows:

Preparation of Dye Solution.—A one per cent solution of rose bengal (Coleman and Bell) in physiologic saline is prepared, filtered through hard, lint-free filter paper and sterilized in an autoclave in 15 c.c. amounts in non-soluble glass bottles twenty minutes at 10 pounds pressure. The bottles are stoppered with alkali boiled, washed rubber stoppers held over the mouths of the bottles during sterilization by a loosely tied on muslin cap. After removal from the autoclave the tops of the stoppers can be grasped through the muslin and the stoppers inserted without contamination. These vials of dye solution have been used two months after preparation without any reactions developing in the patients.

Technic of Test.—Irrespective of body weight, approximately 10 c.c. of the 10 per cent dye solution is injected in an arm vein, using about thirty seconds for the injection. The syringe is quickly disconnected from the needle and 10 c.c. of sterile saline in a second syringe previously filled and ready, is injected through the same needle slowly, so that the syringe is emptied at exactly two minutes from the start of the dye injection. Ten c.c. of blood are then drawn back into the syringe, the syringe and needle withdrawn, and the blood ejected into a centrifuge tube containing a few crystals of potassium oxalate, which is then transferred, as soon as mixed to a vest pocket or other light-free container. Exactly six minutes after the first sample is withdrawn the second sample is drawn, similarly oxalated, and protected from light. This protection is made desirable by the hemolytic action of fluorescein dyes on red blood cells.

The samples of oxalated blood are centrifugated, as promptly as possible, and the plasma removed. Into clear, dry, labeled centrifuge tubes, 3 to 4 c.c. amounts of each of the plasma samples are pipetted and two volumes of acetone added to each and thoroughly mixed. After standing in subdued light for about three minutes the tubes are centrifugated thoroughly and the clear supernatant fluid pipetted off into colorimeter tubes and read, using the first drawn (two-minute) specimen as the standard and the eight-minute specimen as the unknown. We usually pour the specimens back into clean centrifuge tubes, add a drop of 10 per cent sodium hydrate to each, shake, recentrifugate and again read. In some cases the full development of color seems to be helped by this alkalization.

The acetone precipitation of proteins frees the solutions not only from the turbidity present in the oxalated plasma but also from the discoloration due to bile pigments and to traces of hemoglobin due to faulty technic. The resulting solutions are crystal clear, and easily and sharply matched.

CALCULATION

A normally functioning liver removes about 50 per cent of the dye present at two minutes during the subsequent six minutes. Failure to remove this amount is a sign of diminished liver function. Therefore, twice the percentage of dye removed between the first and second samples equals the percentage of liver function. Or, in terms of colorimeter readings, if Rs is the reading of the two-minute or standard sample and Ru that of the eight-minute or unknown sample $200 - \frac{(200\ Rs)}{Ru}$ equals per cent liver function.

RESULTS

These are essentially as previously reported. The test is strictly a test of the liver's ability to remove rose bengal from the blood stream. It is analogous to the phenol-sulphonephthalein test of kidney function in being not a test of any specific type of organ damage, but a measure of the totally remaining functionally active tissue. In this field we believe it the easiest performed and most accurate single laboratory test of liver function.

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DIFFERENTIATION OF THE VARIOUS TYPES OF FATS BY MEANS OF DYES*†

By L. D. Hertert, M.A., San Francisco, Calif.

NILE blue sulphate has been used for many years in England and Europe, but in the main, America's recognition and acceptance has been tardy. However, pathologists maintain that in lipoid studies it is more valuable than either Sudan III or Scharlach R. Its use in the clinical laboratory is almost unknown. It is the purpose of this paper to present the various phases of fat differentiation by means of this stain, with a discussion of new technic.

One of the earlier experimenters with this color agent was Heidenhein,[2] although it remained for later workers to develop its use to a point of practicability. J. Lorraine Smith[8] together with Thorpe[9] presented the most complete analyses of its action and chemical nature, respectively. Lohrisch[5] and Austin[1] first applied it in laboratory medicine while Huepke[3] supplemented the technic by a new method.

Hollande and Cremieux,[4] using nile blue intravitally, have demonstrated that certain granules in the tubercule bacillus stain an intense blue. These granules are neither unique nor specific for this organism and the reaction is probably of little assistance in establishing the identity of the bacillus. Smith, in a series of three articles,[6, 7, 8] clearly defined the behavior of the dye. He applied the reaction to tissue sections and gross mounts of fatty specimens. A brief résumé of these results will serve to establish the nature of the dye-fat equilibrium.

The dye consists of two color bases, a red one (better termed the oxazone) and a blue (the oxazine). The conversion of one type to the other is accomplished by several methods. If alkali is added to a watery solution of the dye, the oxazone is at once in evidence. If dilute sulphuric acid is now added, the blue color base is extracted, and if this extraction is continued, the oxazone is entirely converted to oxazine. The red base (oxazone) may be completely extracted with hot xylol, a process which results in an eosin-pink, fluorescent solution. This extract can be converted to the oxazine type by shaking with dilute sulphuric acid. Moreover, the red base can be diluted into two components, a fluorescent and nonfluorescent fraction. The nonfluorescent portion bears little relationship to our problem and will not be considered further.

With regard to the partition of these bases with fat stuffs, the fatty acids invariably, with exception of oleic, stain a deep nile blue. The mechanism

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is a simple substitution of fatty acid for the sulphate present in the dye salt, and the resulting combination is a color soap. On the other hand, the oxazone or red base stains in a manner similar to either Sudan III or Scharlach R., the intensity of the color depending on the solubility of the dye in the fatty compound. Hence we find neutral fat stained a deep red approaching garnet, the pure fatty acids a deep nile blue, and the intermediary compounds (the partially split fats) a violet-purple or magenta, with their composition indicated by the intensity of shading toward the red or toward the blue. This color range is quite suitable for the examination of stools in that all shades are easily differentiated.

Austin[1] has aptly expressed its use as follows: "As a quantitative estimation of the several amounts of fatty acids, soaps and neutral fats is impracticable in a clinic, we are compelled to form a judgment of the relative proportion of these different forms, much as a practiced eye can detect a leucocytosis by merely glancing at a smear of blood without being actually able to state the grade or extent." Nile blue sulphate has that quality of presenting a complete picture of the deficiency of fat metabolism.

Stool fats are visible in the following colors: neutral fat as the light red or garnet globule, partially split fat as violet or shades af magenta, fatty acids a deep blue while the neutral calcium soaps remain a clear, transparent white. From this reaction it is evident that the nile blue cannot displace the calcium to form a colored soap, and further, the oxazone base is not soluble in this soap. Cholesterol crystals stain as irregular, striated rhomboids with each line rendered more refractile by the nile blue.

Still a third color is perceptible, namely, the light nile green produced by a combination with plant or meat fibers. Although both Austin[1] and Lohrisch[5] report this exceedingly valuable fiber differentiator, it is only rarely that the obtainable commercial products react with these organic remnants to produce the green coloration. It is much more usual to find them a deep blue, and in the case of meat fibers the cellular detail is considerably enhanced. If nuclei are present, their structure is sharply defined.

A slight modification of this green is present in an occasional nest of fatty acid crystals. They are usually needles of palmitic acid mixed with elongated tear-drop types in a quite characteristic arrangement. The inner portions of these nests are always stained a light bluish-green shading into a deep blue at the outer margins.

In practical application, the dye should be kept in neutral or preferably acid solution. Smith[7] after experimenting with a number of acids advised the use of sulphurous. In our experience acetic was quite as satisfactory and is more practical in the laboratory.

A stain of the following composition has been found very useful for stool examination.

Nile blue sulphate*	0.1 gm.
Glacial acetic acid	1. c.c.
Aqua, distilled water	100. c.c.

*Product of National Aniline and Chemical Co.

Let this stand for several days before using. It will keep indefinitely.

Suitable care must be taken in handling the dry powder as it flies about and adheres to every moist surface. It is one of the most tenacious of dyes and will discolor any substance except a metal surface. Test tubes or slides used during these examinations should be kept separate from the laboratory stock. They may be cleaned easily by boiling in lysol.

There is one notable error that may occur, namely, a masking of neutral fats by an over-abundance of fatty acids. Occasionally in stools especially rich in acid crystals there appears such an intense blue that the magenta is not apparent. However, the use of Huepke's[3] technic overcomes this difficulty.

A small piece of stool is mixed with a few drops of a saturated solution of cupric nitrate. This mixture is warmed gently until bubbles form but is never boiled. During the heating period metallic-green copper soaps are formed with the fatty acids. When this phase of the reaction is complete, a drop of 0.25 per cent alcohol dimethyl-amino-azo-benzaldehyde is added. Neutral fat stains a brilliant yellow with the latter reagent. Occasionally the stool is too acid and the red color of the benzaldehyde obscures the yellow fat globules. In this event the stool should be neutralized with a little sodium hydroxide. Huepke[3] advises the use of Orleans as a counterstain in instances of a hyperacidity.

The differentiation of neutral from acid fats by the latter method is clear-cut and accurate. It is possible at times to observe neutral fat globules within the nests of fatty acid crystals. It serves admirably as a check upon those cases requiring it.

A combination of the two technics will lend almost as much information as quantitative examination of stools for the various fat fractions. To the clinician and especially to the pediatrician and internist it gives a test that may easily be carried out and provides a rapid estimation of digestive deficiency.

With very little effort one can become proficient in reading the color complex.

Experiments are under way at the present time to apply these dyes in the estimation of serum lipoids and also the differentiation of fatty and waxy casts.

SUMMARY

1. A routine method of examining stools for fatty acids, neutral fats, neutral calcium soaps, meat fibers, and cholesterin crystals has been discussed.

2. A check method for the accurate differentiation of neutral and acid fats has been included.

3. The use of both reactions has been applied to the examination of the stools.

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DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

Barbiturism: The Separation of Barbituric Hypnotics From the Urine, Biancalani, G. Diagn. E. Techn. di Lab. 2: 19, 1931.

The urine is acidified with dilute acetic acid and evaporated to a syrupy consistency after which it is repeatedly extracted with ether. The combined extracts are then evaporated to remove the greater part of the solvent and the residue allowed to stand until the separation of crystals which are then dried, powdered, dessicated over sulphuric acid and identified by their melting point and chemical characteristics.

Tissue: Serial Sectioning of Small Nematodes, Doubrow S. and Rousset J. Bull. d' Hist. 6: 416, 1929.

The worms are fixed for four to twelve hours in a modified Schaudinn's solution, namely a saturated solution of perchloride of mercury in 80 per cent alcohol with the addition of 5 per cent glacial acetic acid. They are transferred to 80 per cent alcohol tinted with a few drops of iodine to the color of a good madeira wine where they are left for a week on the average, but for longer without damage if desired. After several baths in 90 per cent alcohol (absolute alcohol is not indispensable) they are left for twenty-four hours in butyl alcohol, and again for twenty-four hours in an incubator at 56° C. in butyl alcohol saturated with paraffin. Next follow one, or better, two baths in pure paraffin each lasting twenty-four hours, after which the usual procedure allows of serial sections being fastened on slides and stained.

Flagella Stain: Safford C. E. and Fleisher M. S. Stain Tech. 6: 43, 1931.

The complete method which was found most satisfactory is as follows:

1. Clean slides, slides must be washed in soap and water, dipped in chromic acid, rinsed in distilled water, dipped in acid alcohol, and then thoroughly flamed.

2. Make an emulsion of organisms in distilled water; the emulsion must be very thin (i.e. just perceptibly turbid) and the organisms should be taken from the water of condensation of a ten- to sixteen-hour agar slant culture.

3. Incubate the bacterial emulsion for thirty minutes at 37° C.

4. Place with a pipette a few drops of the bacterial emulsion on the clean slide, spread by tipping or rocking the slide and drain off excess fluid.

5. Dry in air and do not heat.

6. Cover with fixative (fresh) heat to steaming, then allow to act for two minutes. Fixative is prepared as follows:

100 c.c. one-fourth saturated (aqueous) solution of picric acid; 5 gm. tannic acid; 7.5 gm. ferrous sulphate.

NOTE: In case one is dealing with flagella very hard to stain, it may be well to repeat the heating. The fixative will retain its qualities for no more than forty-eight hours.

7. Wash with tap water and dry.

8. Cover slide with Fontana spirochete stain, heat to steaming, then allow to act for one to two minutes. Wash in tap water and dry. Stain must be freshly prepared and is made as follows:

(a) To 25 c.c. of 2 per cent $AgNO_3$ add dilute ammonia until the precipitate which forms, redissolves.

(b) Then add more $AgNO_3$ until a faint turbidity results. A clear solution is useless.

The silver staining appeared to give a far sharper picture than any staining with anilin dyes.

It must, however, be recognized that in any attempt at flagella staining, the essential features after the cleanliness of the slide are the condition of the organisms at the time the spread is made, and the method of making the spread on the slide (the handling of the bacteria and rapidity of drying).

Tissue: A Combined Gram-Pappenheim Stain for Formalin-Fixed Tissues, Scudder S. A., and Lisa J. R. Stain Tech. 6: 51, 1931.

The method which has given excellent results is as follows: Paraffin sections of tissue fixed in 10 per cent formalin are prepared as usual for staining.

1. Flush with crystal violet phosphate solution three to five minutes (the buffer solution is made according to the method of Clark and Lubs).

Crystal violet	1 gm.
Phosphate buffer 6.6 to 7.0	10 c.c.
Aq. dest.	90 c.c.

2. Decant and flush with iodine solution.

Sublimed iodine	2 gm.
N/1 NaOH	10 c.c.
Aq. dest.	90 c.c.

3. Decolorize with Merck's pure technical acetone ten seconds or less. Decant quickly and avoid the slightest drying of the section.

4. Flush the section with counterstain one to two minutes (2 parts 2 per cent aqueous over-ethylated methyl green, with 1 part $\frac{1}{3}$ per cent aqueous pyronin yellowish; allowed to stand several hours before using. Mix well before using.)

5. Wash in tap water. Blot but do not allow to dry.

6. Immerse quickly in oil of origanum Cretic. Differentiate less than one minute. Blot.

7. Clear in two changes of bergamot oil.

8. Blot, mount in balsam. Procedures 1 to 5 should be performed with precision and the slide should be kept moist. The time allowed for counterstain is important since over-exposure removes the stain from gram-positive bacteria. Over-differentiation in oil of origanum Cretic removes the counterstain from Neisserian organisms. Oil of bergamot does not affect the stain even if the slides are immersed overnight.

The special counterstain can be used with the Hucker ammonium oxalate solution in the technic which has been described by Lillie and renders better differentiation than picric acid and fuchsin counterstains recently advocated by Brown and Brenn.

The results are as follows: Gram-positive bacteria are purple black, reversal of the Gram stain has not been noted. The majority of gram-negative bacteria appear faded pink; however, a small influenzal bacillus which has frequently been encountered in exudate, stains deep magenta. Neisserian organisms are deep magenta. The capsule of B. Friedlander stains a transparent lavender. Polynuclear cells have dark blue-purple nuclei and very faint lavender cytoplasm. Small lymphocytes have homogeneous very deep clear blue nuclei; the cytoplasm is unstained or a very faint magenta violet to magenta. The nuclei of plasma cells are dark navy blue with cart-wheel arrangement; the cytoplasm is very homogeneous deep magenta. The eosinophile nucleus is like that of a polynuclear, with granules fairly distinct; staining reddish and occasionally purplish. Endothelial cells have pale blue nuclei; the cytoplasm is light pinkish lavender. Giant cells have nuclei similar to those of the endothelial cells, with the cytoplasm like that of the plasma cell. The red blood cells are bronze to red.

Pneumococcus Typing, Armstrong R. R. Brit. M. J. 1: 214, 1931.

A sample of sputum is chosen and emulsified in a watch glass with an equal volume of broth. Half a cubic centimeter of the emulsion is inoculated into the peritoneal cavity of a mouse. Four hours later the peritoneal contents are sampled by aspiration in the follow-

ing manner: The mouse is grasped by the scruff between the finger and thumb of the left hand; at the same time the animal's tail is firmly held against the inner side of the palm of the hand by the third and fourth fingers. The abdomen is thus conveniently explored by means of a 1 c.c. record syringe with a fine number 18 rustless steel needle attached. The barrel of the syringe should be held by the third and fourth fingers of the right hand against the palm, and the plunger lifted by the right thumb and index finger. Four drops of the peritoneal exudate thus obtained are expressed on slides marked with a grease pencil to correspond with the three chief pneumococcal types and control; the drops are at once mixed with a platinum loopful of types I, II and III agglutinating serums and rabbits' normal serum. The drops are covered with cover slips as soon as mixed, and examined at once under the microscope, a number 4 ocular and a one-sixth inch objective with plane mirror reflector being used, without a condenser. The immediate result, in the case of a positive reaction, is a conspicuous increase in size of the pneumococci, due to conjugation of coccus and homologous antibody. Brownian movement is retarded, and the pneumococci become apparent as lanceolate bodies surrounded by a clear, highly refractive capsular zone. Agglutination, which soon follows, is accelerated if the slide is warmed on the floor of the 37° C. incubator from three to five minutes and then again examined. The end-results of the reaction are the aggregation of masses of agglutinated pneumococci and leucocytes; the pneumococci, sensitized by conjugation with the antibodies, are chemotactic to the phagocytes and polymorphs present in the peritoneal exudate. This characteristic reaction is seen only when virulent pneumococci are effective, belonging to the recognized serologic types. The result, at times, may be a little obscured by the presence of undigested débris from the sputum, if exploration of the mouse's peritoneum is attempted too soon or the initial dose of sputum is too large.

Gastric Acidity, Combined Test For. Matzner, M. J., Gray, I., and Greenfield, H. Arch. Int. Med. **47**: 422, 1931.

The patient was requested to urinate at 6:30 A.M., emptying the bladder as completely as possible. This was done again at 8:30 A.M., and the hydrogen-ion concentration (P_H) of this second specimen was determined immediately.

A Rehfuss tube was passed about 9:00 A.M. and the gastric contents of the stomach during fasting extracted. The stomach was washed with water until a clear return was obtained. The patient was then requested to drink two cups of warm Steero bouillon (one cube to a cup of warm water). Gastric extractions were made from every twenty to thirty minutes over a period of from three to five hours. The patient was instructed to empty the bladder as completely as possible at each urination, and was kept at comparative rest throughout the entire test. The gastric specimens were quantitatively analyzed for free and total acidity.

The hydrogen-ion concentration of the urine was immediately determined with the Hellige comparator. A determination can be made in a few minutes. This method is extremely simple and lends itself readily to clinical use.

The method is useful in suggesting the presence or absence of free HCl by a study of the urinary alkaline tide.

Galactose, In Tests of The Function of the Liver, Shay H., Schloss E. M., and Bell M. A. Arch. Int. Med. **47**: 301, 1931.

The authors, from their studies, believe that galactose is suitable for the testing of liver function because:

1. It is obtainable in pure form.
2. It is readily absorbed from the digestive tract.
3. It is converted into glycogen by the liver with some difficulty as compared to other sugars (dextrose and fructose).
4. It is practically not utilizable by any other tissues than the liver.
5. After it reaches the general circulation, it is excreted in the urine regardless of either the state of the renal excretory mechanism or the activity of the endocrine glands.

Blood Sedimentation: Prognostic Value In Arthritis, A Modification of Technic, Weiss A. Am. J. Med. Sc. **181**: 379-392, 1931.

By venipuncture 1.6 c.c. of blood is drawn into a syringe containing 0.4 c.c. of 3.5 per cent solution of sodium citrate. The piston of the syringe is then drawn back slightly and its contents mixed thoroughly. The mixture is then emptied into the sedimentation cylinder. At the end of forty-five minutes a reading is made. This cylinder is constructed to hold 2 c.c. The calibrated column is 113 mm. long, 5 mm. inner diameter, and is subdivided into 100 equal parts. Thus the readings can be made directly in percentage. As a rule a fine capillary tube slightly longer than the cylinder is put inside and the blood mixture emptied alongside it. This ensures perfect mixing and facilitates filling and emptying of the cylinder. This tubing is removed gradually as the blood column rises in the calibrated tube. The normal reading is 3 to 5 per cent in forty-five minutes.

The conclusions drawn from a study of 150 cases of infectious arthritis follow:

1. The sedimentation rate was high in rheumatic fever and did not return to normal with the disappearance of the arthritic symptoms. The return to normal of 3 or 5 per cent did not occur until three to seven weeks after the disappearance of complaints.

2. Severity of the joint manifestations was not accompanied by a proportionately high sedimentation rate.

3. Sedimentation rate did not return to normal any faster in mild cases than in the more severe.

4. As far as the sedimentation rate gives evidence, cases of acute articular rheumatism do not tend to become chronic.

5. Cases of acute rheumatic fever are not to be discharged before the sedimentation rate reaches normal.

6. The sedimentation in these cases is not due to the amount of anemia that is always present.

7. The sedimentation rate in cases of infectious arthritis is very high (60 to 80 per cent) and usually does not return to normal, unless the cause can be discovered and removed.

8. This procedure does not differentiate the various types of secondary arthritis such as tuberculous, gonorrheal, or acute arthritis deformans.

9. The high sedimentation rate is not due to the anemia that is usually present.

This procedure is of no diagnostic value in arthritis, but is of great help in prognosis and an essential guide in treatment and convalescence. No rheumatic fever should be discharged from close observation until the sedimentation rate has reached normal. Any case that simulates acute rheumatic fever but which shows no tendency for the sedimentation rate to gradually return to normal can be understood to be one of secondary arthritis.

Hemolytic Streptococcus Carriers, Improved Method for Detection of, Mueller J. H., and Whitman L. J. Bact. **27**: 219, 1931.

A throat swab, inoculated in the ordinary way from the tonsils and back of the throat, is inserted immediately into a test tube containing 1.5 c.c. of ordinary extract or infusion broth, and 0.3 c.c. of a 1 per cent solution of anhydrous Na_2CO_3. The carbonate is added by means of a sterile pipette after the broth has been autoclaved. The swab is allowed to soak in the alkaline broth from one to two hours at room temperature, or may be incubated at 37° C. for half an hour. It is then stirred around in the tube, and a good sized drop transferred with the swab to the surface of one of the peptone blood agar plates described below, and spread evenly over the surface with a capillary or wire spreader. After twenty-four hours' incubation, hemolytic colonies are clearly visible, and may be fished and streaked out for isolation on fresh blood agar plates.

The medium used for plating is as follows:

Peptone (Parke, Davis)	1 per cent
Sodium chloride	0.8 per cent
Agar	1.5 to 1.8 per cent

Dissolve in water, titrate to P_H 7.6 and add 10 per cent of horse blood.

Antiseptics, Determination of the Value of, Allen A. W., and Wright I. S. J. A. M. A. **96**: 920, 1931.

The following method is described as testing the penetrating power of an antiseptic and its ability to act in the presence of blood serum.

Standard beef extract agar, 250 c.c., in flasks is cooled to a point at which it will not cause coagulation of the blood serum. Blood serum is then added to make 10 per cent concentration. The flasks are immediately inoculated with a twenty-four-hour broth culture of the test organism. The inoculation is made by first diluting the culture 1:20,000 and then adding 1 c.c. of this dilution to the flasks of medium. Immediately after inoculation and mixing by careful rotating, 30 c.c. of the inoculated mediums are then transferred to Petri dishes. After the agar is allowed to cool and form a solid jelly, a disk having a diameter of 2 cm. is removed from the center of the Petri dishes. The cup or depression thus formed is then sealed at the bottom with a few drops of liquid agar. Originally glass stoppers were used to produce the desired cup. This was found a bad practice, causing injury to the medium at the time the glass stopper is removed. A nickel-plated, specially sharpened cork-borer, sterilized in a free flame, has been found most efficient in easily removing the disk from the center of the agar plate. After the medium is allowed to cool and form a solid jelly, 0.5 c.c. of the antiseptic or disinfectant to be tested is placed in the cup at the center of the Petri dish. If the antiseptic is to be applied for only a short-time period in practice, the technic of the test is to remove the antiseptic by means of sterile filter paper after five, ten, fifteen, or thirty minutes in the cup. If the method of application recommended indicates that the product would be in constant application with the tissues over a period of time, such as results in the application of wet dressings, antiseptic powders or ointments, the antiseptic or germicide is allowed to remain in the cup for a period of forty-eight hours. The Petri dishes, after treatment with the antiseptic or after its removal from the cup or depression at the center, are incubated for a period of forty-eight hours at a temperature of 37° C., for the purpose of securing growth of any organisms not inhibited by the antiseptic or germicide. The results are tabulated in distances from the cup, showing no growth. A further test is then made to determine whether or not the action observed was inhibitive or germicidal. Small disks (0.1 c.c.) are removed from the mediums at distances of 0.1 cm. each, until the area where there is visible growth is reached. The disks are transferred to standard beef extract broth in sufficient volume to avoid any inhibitive action, and after breaking up in the mediums are incubated for a further period of forty-eight hours. The results are tabulated, indicating the distances from the cup where the germicidal action was evident from the fact that no growth of the test organism was obtained.

Organisms of known virility and resistance to phenol according to the classification of Reddish are used. They are transplanted for three successive days on agar slants of the following composition: Armour's peptone, 10 gm.; Liebig's beef extract, 5 gm.; salt, 5 gm.; agar, 15 gm.; distilled water, 1,000 c.c., adjusted to a P_H of 6.8. Five cubic centimeters of the agar is placed in each of the usual ⅝ by 5⅞ inch lipped test tubes and autoclaved at 20 pounds pressure for one-half hour. Immediately on removal from the sterilizer the tubes are set at an angle of about 15 degrees to the horizon to insure as nearly as possible even and equal slant surfaces, and also to prevent the formation of the undesirable water of condensation which results from the usual remelting and recooling method of preparing the slants.

From the third successive culture a fresh subculture (known as A) of each organism to be used, is made as follows: With a straight nichrome needle at least three streaks are made on a fresh agar slant (A). This is incubated at 37.5° C. for twenty-four hours. At the expiration of this time, 10 c.c. of the antiseptic to be tested is added to the culture

(A). This should be adequate to cover the slant completely when in an upright position. The antiseptic is added at a temperature of 37.5° C. Thenceforth the cultures are maintained at the same temperature by means of a water-bath. At intervals of 5, 10, 15, 30 and 60 minutes, transplants from the cultures (A) are made on fresh agar slants (B) or Petri dishes (Mediums as previously described) as follows: With a straight nichrome needle a transplant is removed from the surface of the agar slant (A) and at least three streaks made on the fresh agar (B). This (B) is incubated at 37.5° C. for forty-eight hours and then read.

Subcultures (C) are made from all transplants (B) in which there is doubt as to the presence of growth of the organisms.

It is possible to change the time of transplanting from A to B according to the antiseptic used and the accuracy desired. The killing time can be determined more accurately by shortening this transplant time.

This method simulates the presence of a massive infection.

Tissue: Method for Decolorizing Preparations Impregnated by Cajol's Photographic Method, Branceson A., and Campos R. Bull. Soc. Ital. Biol. Sper. 5: 780, 1930.

(1) Remove paraffin; (2) pass through alcohol; (3) place in Lugol's solution until silver disappears; (4) wash in distilled water; (5) place in 5 per cent sodium sulphate until brown color disappears; (6) wash thoroughly in tap water; (7) stain as usual with H and E.

Malaria, A Rapid Method for Giemsa's Stain, Neri F. Rio di Malariologia 9: 150, 1930.

By mixing Giemsa's fluid with four times the quantity of methyl alcohol a product is obtained which will keep its properties for fifteen days if placed in a yellow glass bottle. This is applied to the smear for a minute, then double the quantity of neutral distilled water for five minutes, after which the slide is washed in tap water.

Flagellates, A Simple Solidified Hemoglobinized Saline Agar Medium for Surface Cultures of Leishmania and Allied Flagellates, Row R. Indian Med. Gaz. 65: 319, 1930.

In each tube place 6 c.c. of an ordinary 3 per cent agar in normal saline. When required the agar is melted and to each tube is added an equal volume of hemoglobinized saline. After mixture, the tubes are sloped and when the medium is solid it is incubated in the upright position at 37° C. for some hours and then in the ice box. The liquid of condensation is then removed carefully with a pipette and kept sterile for subsequent use. The tubes are left with the surface of the medium uppermost in order to obtain a dry surface, which is then inoculated at some distance from the bottom with a drop or two of culture. The tubes are again left till the surface appears dry, after which 1 to 2 c.c. of the condensation fluid are introduced into the bottom of the tube, care being taken not to moisten the surface of the medium. The tubes are then incubated in the slanting position with the surface downward. By this method the rich cultures which are obtained in the Petri dish are secured without difficulty.

REVIEWS

Books for Review should be sent to Dr. Warren T. Vaughan, Medical Arts Building, Richmond, Va.

*The Pathology of Internal Diseases**

IT IS unfortunate that so many regard pathology as a specialized study, distinct and apart, to be associated only with the museum, the morgue, and the laboratory.

The fallacy of this view is obvious from the fact that the really great physicians and surgeons are characterized by a clear conception of disease as a whole, that is, the ability to correlate the clinical signs with the underlying mechanisms which produce them.

As the preface of this book says, "A sound understanding of pathological processes will endow the physician with a roentgen-ray vision, so that the coverings of the body are rolled away and the hidden processes of disease stand revealed."

Dr. Boyd has attempted to do for the pathology of internal diseases what he has previously done for the pathology of surgical conditions: to present the subject in such a fashion that the relation of symptoms to lesions should be paramount. Even the most cursory perusal suffices to show that this objective has been consistently attained.

Dr. Boyd has written a pathology, not merely for the student or the pathologist, but a pathology for the physician in general.

Here is a book which deserves a hearty and enthusiastic welcome for it is not only well but entertainingly written. The style is clear; the phrases are simple and well turned; the word pictures are vivid and graphic; in short, the book is not only thorough and authoritative, it is interesting throughout.

Pathology, as it is here presented, is not a dry array of facts, but a living story of disease, its concomitants and its sequelae. The correlation of symptoms and lesions, which is the thread throughout the book, binding altogether, is fascinating reading.

We do not believe there is anyone in the field of medicine to whom it will not appeal or by whom it should not be read. Nor do we believe that any can read it without sustained interest or continued profit.

The illustrations are excellent throughout and the format characteristic of the work of this publisher.

Both Dr. Boyd and the publishers are to be congratulated upon a work of real value to the physician.

High Frequency Practice†

UNLESS the practitioners and students for whom this book is written have had some advanced work in electricity, the chapter on the physics of high-frequency currents will present many difficulties. Some definitions are hazy, and explanations involved; and much that is presented in useless.

*The Pathology of Internal Diseases. By William Boyd, M.D., Professor of Pathology in the University of Manitoba; Pathologist to the Winnipeg General Hospital, Winnipeg, Canada. Octavo, 888 pages with 298 illustrations. Cloth. Lea and Febiger, Philadelphia, Pa.

†High Frequency Practice for Practitioners and Students. By Burton Baker Grover, M.D., Author of Handbook of Electrotherapy; President of Western School of Physical Therapy; President of the Western Electrotherapeutic Association, 1919-1920, etc. Illustrated with Engravings. Thoroughly Revised and Rewritten. Ed. 5, 1928. Cloth. Kansas City. The Electron Press, 1928.

NOTE: In so far ar practicable the book review section will present to the reader (a) interesting knowledge on the subject under discussion, culled from the volume reviewed, and (b) description of the contents so that the reader may judge as to his personal need for the volume.

We trust that the scientific information printed in these pages will make the reading thereof desirable per se and will thereby justify the space allotted thereto.

Errors of all sorts are abundant throughout the book. What purport to be sentences often turn out to be phrases and clauses. Contradictions and repetitions are not infrequent. Diameters are measured in two dimensions.

Much of the contents might profitably be omitted. The chapter on blood pressure, consisting of forty-four pages, eleven of which are devoted to treatment, could be condensed to one-third that number without loss. An article by another writer, that was published in a journal several years ago and that has appeared in reprint form very frequently since, is set down bodily (with the author's consent). These are but examples.

The most useful portion of the book is the last half wherein the author follows, though in synoptic fashion, the order pursued in textbooks on theory and practice, substituting physical therapy for the usual medical treatment, the words, physical therapy, being used advisedly for the author does not confine himself to high-frequency currents. The appendix should not be overlooked.

It is stated on the title page that the book has been thoroughly revised and rewritten. Repetition of these to better effect might enhance the value for the reader after encountering the so very numerous mistakes of all kinds, unconsciously searches for more and thus overlooks much that might be of worth.

*Problems and Methods of Research in Protozoology**

THIS book should receive a hearty welcome, not only from those whose primary interest is in protozoology, but also from those laboratory workers into whose field problems in protozoology may occasionally enter.

It is the conjoint product of twenty-seven specialists in this field ably coordinated by the editors whose contributions to protozoology need no comment.

The book appeals to the student as well as to the seasoned investigator. Its purpose was to present under one cover important recent developments in protozoologic research which have been scattered through the literature, as well as important contributions hitherto unpublished; to present recent and new methods, and, especially to suggest and point out problems of importance and practical value which demand investigation.

The growing appreciation of the importance of protozoology in medicine renders this volume both timely and exceedingly useful for the contributions are eminently practical and present many and varied phases of the subject. The text can be regarded as entirely authoritative.

There is an excellent index and also a full bibliography including, also, a list of recent books devoted in whole or in part to protozoology, as well as a similar list of Journals.

Introduction to Physiological Chemistry†

PHYSIOLOGY, like medicine as a whole, is, as it were, in a state of flux, the concepts of today often requiring drastic revision on the morrow.

This new second edition of a work previously accorded a very favorable reception has been rewritten and reset to bring it in accord with recent advances.

It deserves, and will doubtless receive, as cordial a reception as its predecessor.

Applied Physiology‡

THIS is the third edition of a very useful book, the purpose of which is to emphasize the clinical correlation of physiology and disease.

*Problems and Methods of Research In Protozoology. Edited by Robert Hegner, Professor of Protozoology and Justice Andrews, Associate in Protozoology, Johns Hopkins University. Cloth, 532 pages, 32 figures. The Macmillan Co., New York.

†Introduction to Physiological Chemistry. By M. Bodansky, Professor of Pathological Chemistry, University of Texas. Cloth, 542 pages, 46 figures. John Wiley and Sons, Inc., New York.

‡Applied Physiology. By S. Wright, Professor of Physiology, University of London. Cloth, 552 pages, 128 figures. Oxford University Press, New York.

To this end preceding each chapter there is a synopsis in which those parts of the subject which are wholly or mainly of clinical interest are indicated by italics. This, together with a very complete index greatly increases the usefulness of the text.

Without being encyclopedic, the book is very comprehensive. It is an authoritative, clear, and eminently practical presentation of an important subject of great clinical value.

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*Elementary Zoology**

THIS volume, based upon the author's *Manual of Zoology*, presents a clear and sufficiently comprehensive review of the subject for those who wish to review their working knowledge.

The fact that a second edition has become necessary is an indication of its usefulness to those to whom it is addressed.

Minor Surgery and Bandaging†

THIS, the twentieth edition of this well-known and exceedingly useful little book has been amply revised and includes such recent developments as the injection treatment of varicose veins.

All the procedures are clearly described in detail and there are ample illustrations to aid the test.

The volume, small and compact, should be an exceedingly useful reference work for the practitioner at large and can be highly recommended.

Manual of Physiology‡

THIS third edition of a well-known and exceedingly useful text has been carefully revised in order to bring it up to date.

It will undoubtedly be as widely read in the future as it has been in the past.

Handbook of Protozoology§

THE advances of modern medicine have not only emphasized the importance of protozoan parasites as a cause of disease but have made it necessary for all who are concerned with the study of disease to have, at least, a working knowledge of these important forms.

The purpose of the present volume is not to supply an encyclopedic compendium on the subject but rather to serve as an introductory handbook of information on the subject; to furnish in a succinct way such information as will conduce to a satisfactory working comprehension of the representative free-living and parasitic protozoa.

The second section which constitutes the greater portion of the book (392 pages) is devoted to brief but satisfactory descriptions of the common and more important forms.

The illustrations of which there are nearly 1,500, are well done and equally well reproduced. There is an appendix covering the technical methods useful in the study and cultivation of protozoa.

Both author and publisher are to be congratulated upon a very useful volume.

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*Elementary Zoology For Medical Students. By L. A. Borrodoile, Sc.D. Lecturer in Zoology, Cambridge University. Cloth, 397 pages, 251 figures, ed. 2. Oxford University Press, New York.

†Minor Surgery & Bandaging, for the use of House Surgeons, Dressers, and Junior Practitioners. By Groynne Williams, Surgeon University College Hospital. Cloth, 445 pages, 262 illustrations. F. A. Davis Co., Philadelphia, Pa.

‡Manual of Physiology. By H. W. Lyle, Lecturer on Physiology, King's College, London, and David De Lanza, Lecturer on Physiology, King's College, London. Cloth, 820 pages, 138 figures. Oxford University Press, New York.

§Handbook of Protozoology. By Richard R. Kudo, D.Sc., Assistant Prof. of Zoology, University of Illinois. Cloth, 451 pages, 175 etchings, containing 1,463 figures. Charles C. Thomas, Springfield, Ill.

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EDITORIALS

Sophistication of Vaccines

THOSE who have read the Symposium on Arthritis which appeared in the August and September, 1930, numbers of *The Journal* will undoubtedly have been impressed by the observation that those writers who feel that they have been successful in isolating a specific causative organism for chronic arthritis, believe that the specificity is of very high grade. Both Crowe and Small have demonstrated the effectiveness of extremely small doses of their vaccines and have cautioned against the indiscriminate use of large dosages. If we interpret their observations correctly, the bacterial factor appears to be associated with a condition of hypersensitiveness or allergy and the treatment should therefore be described as a desensitization treatment rather than an immunizing treatment.

Those who have had any large experience with either the Crowe vaccine or the Small vaccine have learned from experience that in a sensitive individual large doses may considerably aggravate the symptoms instead of ameliorating them. Certainly experience has shown that the dosage must be small, even minute, that it must be very carefully graded and that the treatment must be individualized depending upon the response to the injections.

The authors have, through years of study, carefully worked out and established their technic and have emphasized the extreme importance of strict adherence to their routines if successful results are to be obtained.

All who are interested in the arthritic problem must necessarily hope that such observations and studies will eventually help solve it. The work is promising but it is open to question whether it has reached such a stage that it can be applied successfully by any doctor following the reading of a few printed instructions and without considerable experience and understanding of the problem in hand. Since even among those whose primary interest is in arthritis there is not yet a universal agreement as to the value of these vaccines, and since much more research remains to be done, there may be a serious setback to the progress of these investigations if attempts are made to apply the method indiscriminately, in a haphazard manner, and without carefully following the directions outlined by their originators.

Those who have read the contribution of Doctor Crowe and his *Handbook of the Vaccine Treatment of Chronic Rheumatic Disease* cannot but be impressed by the author's enthusiasm and his conviction that if his technic is carefully followed good results may be obtained in a fair proportion of cases. While he uses a pooled vaccine at times, he at the same time emphasizes the specificity of the reaction and the frequent need for concurrent use of autogenous vaccine.

There has recently become available in this country, manufactured by one of the biological supply houses, a vaccine containing Crowe's organisms, concerning which the manufacturer quotes from Crowe's report and gives Crowe's statistics of results. While this vaccine does contain Crowe's organisms it also contains pneumococcus and the colon bacillus neither of which are in the vaccine prepared by Crowe and the dosage recommended by the manufacturer far exceeds that specified by the London investigator. We presume that the manufacturer has failed to understand Doctor Crowe's use of a stock vaccine and has interpreted this as an indication that he believed the response to be nonspecific. This as we read Doctor Crowe's communication is the exact opposite of his conception. However, this appears to be the only reason or excuse for incorporating into the vaccine such other nonspecific organisms as the pneumococcus and the colon bacillus.

The tremendous dosage recommended by the manufacturer in contrast to that recommended by Crowe would indicate that the former has failed to realize the different significance between bacterial desensitization and bacterial immunization, for the dosage outlined follows that customarily recommended for immunizing vaccines.

The medical profession has succeeded pretty well in stopping the practice among druggists of substituting "something just as good" for drugs specified in prescriptions. It may be that similar steps will have to be undertaken with respect to biological products. It was for the purpose of preventing such situations that insulin and similar preparations were patented. In this way their manufacture and distribution can be satisfactorily controlled. The case in hand appears to offer justification for this policy of patenting medical discoveries.

At any rate those who may have occasion to use a vaccine purporting to contain Crowe's organisms should realize that at the present time they may be using a preparation of altogether different composition. And in the event they cannot obtain the results which Crowe describes they will know a possible reason therefor.

—*W. T. V.*

All Hail Phyone, the Newest Hormone

WHEN God saw fit to modify the social metabolism in the Garden of Eden he mobilized the undesirable elements[1] and brought about their elimination so that Eden thereafter might be a better place in which to live. And he placed hormones, or messengers, in the form of cherubims with a flaming sword at the east of the garden to see that no further dietetic or endocrine errors occurred within. Ever since that time the whole physical, intellectual, moral, and spiritual life of man has been modified or controlled by the influences, good, bad, or indifferent, of his glands of internal secretion. With an action often obscure, indefinite, and unsuspected, it has been only within the more recent decades that man's knowledge of what these glands may really do to him, or for him, has advanced very much beyond that of Adam's rather hastily formed conclusions. And there are apparently a good many natives still left who are willing to pay a substantial price for the effects which they hope various extracts, juices or gravies made from one or more of these glands may produce. Even the religion handed down to us from the Middle Ages and later centuries has been tinctured a good deal by the great artists of the times who often endowed their angels and semicelestial beings with the blessings not only of rickets but of endocrine dystrophies as well—sacred favors apparently frequently denied to the common herd.

Since the days of Brown-Sequard some of our greatest scientists and some of our shrewdest commercial exploiters, have devoted themselves most assiduously to the study of these glands and their possible influences, direct or indirect, upon man. And these efforts have been attended with a good deal of success in both directions. The rather recent work of Dr. Kamm and his associates on pitressin and pitocin, and of Collip on the parathyroids, are splendid examples of scientific accomplishment not to mention the older work on epinephrine, thyroxin, insulin, the active principles of the gonads, etc. And now from the pharmacologic laboratory of the University of Chicago we have the announcement of further progress in the study of internal secretions. For in a splendid piece of work by Van Dyke and Wallen-Lawrence[2] a method has been developed for preparing a potent growth-promoting extract of the anterior lobes of beef pituitary bodies, and this extract is suitable for clinical use. It appears to be free from any luteinizing principle and has been thoroughly assayed biologically by its growth-promoting action on both normal and hypophysectomized rats. Van Dyke and Lawrence have named this hormone *phyone* (*φύω*, I cause to grow). And now with a preparation of phyone suitable for clinical use available we may well expect notable progress to be made in the study of the action of this hormone in the human

species at an early date. Thus a more adequate knowledge of the true function, or functions, of the *pars glandularis* of the hypophysis seems now to lie only a little way beyond our intellectual horizon. The pharmacologic laboratory has carried forward one step further the work originally started by Evans and Long[3] and nurtured by Putnam, Teel and Benedict,[4] Teel,[5] and others.

Almost simultaneously with the Chicago announcement comes a preliminary communication by Thompson[6] from King's College, London, stating that an *anti-growth factor* has been extracted from fresh parathyroid glands. The glands are extracted for forty-eight hours with 85 per cent alcohol containing 15 per cent sulphuric acid. The filtrate is then neutralized, concentrated and filtered again. When this extract was injected subcutaneously into 42 rats it was found that at the end of sixteen weeks the average weight of these rats was only 92 grams as compared with 157 grams which the controls weighed. And similar experiments carried out on rats inoculated with Jensen rat sarcomas indicated that the growth of these tumors was also much affected by the extract. This hormone, if such it be, is so far unnamed.

If it can be proved that an endocrine extract such as this can inhibit, or prevent, the growth of cancers it will be only a little while until the active principles of the glands of internal secretion will begin to assume an importance to the human race which can be equaled only by some such substance as rubber. Meanwhile we pause and wait for our intellects to stretch so that they will encompass the conception that our endocrine glands may produce two substances, one which makes us big and one which makes us little. And a man's avoirdupois may depend largely on how these two "elements are mixt" in him.

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—*D. E. J.*